REVIEW ARTICLE

Tumor Necrosis Factor Ligand Superfamily: Involvement in the Pathology of Malignant Lymphomas

By Hans-Jürgen Gruss and Steven K. Dower

PHYSIOLOGIC and pathologic activities of cytokines are mediated by binding to cell surface receptors (R). Sequence analysis of cytokine receptors defines several subfamilies of membrane proteins with specific homology of functional domains. Receptor subfamilies of related proteins form (1) the Ig superfamily (eg, interleukin-1 receptors [IL-1Rs], fibroblast growth factor (FGF) Rs, platelet-derived growth factor (PDGF) Rs, c-kit, c-fms, flt-3) characterized by varying numbers of Ig-like repeats in the extracellular domain; (2) the hematopoietin (cytokine) receptor superfamily (eg, erythropoietin receptor [EPOR], growth hormone receptor [GHR], prolactin (PRL) Rs, mpl, ciliary neutrophic factor (CNTF) Rs, leukemia-inhibitory factor receptor [LIFR] gp130, IL-2R β and γ chain, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, granulocyte colony-stimulating factor receptor [G-CSFR], and granulocyte-macrophage–CSFR [GM-CSFR]) with conserved cysteine residues and the characteristic WSXWS motif; and (3) the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily based on cysteine-rich repeats in the extracellular region.[1-5] Further, receptors can be functionally, based on signaling, subdivided by common receptor subunits involved in forming several multimeric receptor complexes such as the gp130-associated proteins for IL-6R, LIFR, OncoMR, CNTFR, and IL-11R; common β-chain–associated molecules for IL-3R, IL-5R, and GM-CSFR complex; and common γ-chain–associated members (IL-2R, IL-4R, IL-7R, IL-9R, IL-13R, and IL-15R).[6]

The TNF/NGF receptor superfamily contains at present 10 different membrane proteins and several viral open reading frames encoding TNFR-related molecules. The p75 low-affinity NGF receptor was the first cloned receptor of this family.[7] TNF was originally discovered by its antitumor activity in mice.[8] Subsequently, cloning of two specific receptors for TNF showed that there were related to the NGFR.[9-11] In recent years a new type-I–transmembrane TNF/NGF receptor superfamily has been established.[1,2,4,5] The TNF/NGF receptor superfamily includes the p75 NGFR,[7] p60 TNFR-I,[9-11] p80 TNFR-II,[10] TNFR-RP/TNFR-III,[12] CD27,[13] CD30,[14] CD40,[15] 4-1BB,[16] OX40,[17] and FAS/APO-1.[18,19] In addition, several viral open reading frames encoding soluble TNFRs have been identified, such as SFV-T2 in Shope fibroma virus[20] and Va53 or SaIF19R in Vaccinia virus.[21,22] These receptor superfamily is characterized by multiple cysteine-rich domains in the extracellular (amino-terminal) domain, which have been shown to be involved in ligand binding.[2,10,23-25] The average homology in the cysteine-rich extracellular region between the human family members are in the range of 25% to 30%.[5,26] The NGFR, TNFR-I, TNFR-II, and FAS/APO-1 have a broad tissue distribution, whereas the others (CD27, CD30, CD40, 4-1BB, and OX40) are mainly restricted to cells of the lymphoid/hematopoietic system.[4]

Ligands for these receptors have been identified and belong to two recently formed cytokine superfamilies. The neurotrophins (NT; NGF ligand superfamily) are basic, NGF-like dimeric soluble molecules and include NGF, BDNF, NT-3, NT-4, and NT-5.[27,28] The ligands of the TNF ligand superfamily are acidic, TNF-like molecules with approximately 20% sequence homology in the extracellular domains (range, 12% to 36%) and exist mainly as membrane-bound forms; the biologically active form is a trimeric/multimeric complex. To this group belong TNF,[29-32] LTα,[33,34] LTβ,[35] CD27L,[36] CD30L,[37] CD40L,[38-42] 4-1BBL,[43,44] OX40L,[45,46] and FASL.[47-50] Soluble forms of the TNF ligand superfamily have only been identified so far for TNF, LTα, and FASL.[29,47,51,52] These proteins are involved in regulation of cell proliferation, activation, and differentiation, including control of cell survival or death by apoptosis or cytotoxicity.[4,5]

Malignant lymphomas are a heterogenous group of lymphoid tumors that mainly arise from the lymphoreticular system and are grouped, based on morphologic criteria, into two large categories, Hodgkin's disease (HD) and non-Hodgkin lymphomas (NHL).[53] Furthermore, lymphomas are grouped into three major phenotype categories: B cell, T cell, and HD. The origin of the lymphoma cells in NHLs is most commonly from B cells (90%) and less frequently from T cells (10%), with massive clonal expansion of the malignant cell population. In contrast, HD is defined by common clinical and pathologic feature.[54-56] The diagnosis of HD is typically based on a disrupted lymph node architecture and the presence of the malignant mononucleated Hodgkin and multinucleated Reed-Sternberg (H-RS) cells embedded in an abundance of normal, reactive cells (eg, lymphocytes, histiocytes, eosinophils, plasma cells, and stromal cells) without malignant transformation.[57] HD contains only a low proportion of the neoplastic H-RS cells, accounting for usually less than 1% to 2% of the total tumor cell mass.[54,58-60] The etiology of HD and the origin of the H-RS cells remains unclear with almost every cell type having been described as a normal counterpart.[61-63] Primary and cultured H-RS cells express a heterogenous panel of cytokines and cytokine receptors that correlate with the typical clinical and pathologic presentation of HD cases.[56,64] Cytokines and a cell contact-dependent activation network are critical elements in the pathology of HD.

This review will summarize recent data on the functional

From the Department of Biochemistry, Immunex Research and Development Corp, Seattle, WA.

Submitted November 3, 1994; accepted February 28, 1995.

Address reprint requests to Hans-Jürgen Gruss, MD, Freie University Berlin, UKRV-RRK, Department of Medical Oncology and Applied Molecular Biology, Lindenberger Weg 80, D-13122 Berlin, Germany.

0006-4971/95/8512-0041$3.00/0

role and pathobiologic involvement of the TNF/NGF receptor and TNF ligand superfamilies for the pathology of malignant lymphomas.

STRUCTURAL FEATURES OF TNF RECEPTOR AND LIGAND SUPERFAMILIES

The TNF receptor superfamily consists, at present, of 10 mammalian proteins.[4,10,37,65] In addition, several viral open reading frames (ORFs) have been identified that encode acquired soluble TNF receptors (eg, SFV-T2, va53, SalF 19R, MYX-T2, G4R, and crmB).[10,20-22,66-70] The TNF ligand superfamily contains nine members, ie, the counterparts of the receptors.[5] The ligand for the low-affinity NFG receptor is structurally unrelated to the TNF superfamily of ligands.[27]

The crystal structures of TNFRI (p60), TNF, and LTα have been solved[71-73] and the mechanism of receptor-ligand interaction was illustrated.[73,74] The ligand is a trimer.[71,72] The receptor extracellular ligand binding region is a rod-like structure in which the four cysteine-rich 40 residue repeats each folds tightly together and forms intimate longitudinal contacts with its neighbors. The complex contains one LTα/TNF homotrimer and three receptor chains.[73] The receptors bind in three grooves in the ligand trimer formed by the subunit interfaces; thus each receptor makes contact with two subunits. The structure predicts that the binding of ligand will crosslink three receptors together into a cluster. Recent reports suggest that, for LTα, this model may be an oversimplification. A second form of lymphotoxin (lymphotoxin-β [LTβ] or p33) has been identified[35,75,76] that unlike LTα, appears to be membrane anchored. The TNFR-related protein (TNFRP) has been shown to be a specific receptor for LTβ and hence is TNFRIII.[77] In addition, it appears that LTα and LTβ, when expressed in the same cell, can form heterotrimeric complexes.[76] Because the sites for receptor binding lie at the subunit interfaces, such heterotrimers can contain three different sites (α_2, β_2, and $\alpha\beta$) in various combinations depending on subunit stiochiometry. In which way such heterotrimeric ligands interact with cells expressing combinations of TNFRI, TNFRII, and TNFRIII needs to be clarified.

The overall structures of two TNFR-like viral gene products, the TNF receptors and the ligands, are shown in Fig 1. In addition, Tables 1 and 2 summarize the sizes of the proteins as lengths of the various sequence segments and chromosomal location. Sequence alignments of the extracellular regions of the receptors show that they are distantly related (25% to 30% in general; summarized in Table 3). However when sequence conservation patterns are examined, two features are immediately apparent.[4,10] First, there is a characteristic pattern present in all the sequences, with the majority of conserved positions containing an unusually large number of cysteine residues. Second, all of the proteins are composed of several repeats of a core domain of 30 to 40 residues.[4,10,25] The TNFRI crystal structure shows that this domain is composed of three elongated strands of residues held together by a twisted ladder of three disulphide bonds[73]; the strands that form the core of the structure contain approximately 25 residues, of which 6 are cysteines. The strands are joined by loops of less-conserved structure. Thus, the three-dimensional structure shows that the cysteine-rich repeats in the sequence present the charcteristic structural domain in this family. TNFRI contains four such domains stacked longitudinally to form a bent rod. This rod structure thus forms an extended ligand binding unit, with the ligand contact side chains being located in domains 2 and 3. The largest variation in structure between the family members is found in the region between the C-terminus of the membrane proximal domain and the transmembrane region. This segment varies from 8 residues in FAS[18] to 70 residues in CD27[13] and is presumed to form a variable spacer between the ligand binding unit and the membrane. CD30 has an extracellular region in which the cysteine-rich repeat unit has been duplicated.[14] The observation that the CD30a and CD30b regions are far more closely related (50%; see Table 3) than any of the other family members are, suggests that this structure has arisen from tandem duplication event more recent than those that gave rise to the various family members from a common ancestor. CD30a is as related to the rest of the family as any of the other members, but CD30b, the membrane proximal region, is the most divergent sequence of the family (Table 3). It seems likely therefore that it has evolved rapidly away from the rest of the family, may well have lost ligand binding activity, and serves the purpose of an extended spacer.

It is striking that this family of molecules shows a relatively low level of sequence conservation despite in all likelihood sharing a common fundamental structure. This finding suggests that the sequences have diverged rapidly. In support of this notion, comparisons of the sequences of the same member from different species show unusually low levels of conservation. This finding can be seen from two examples included in Table 3, ie, OX40 and CD40. In both cases the human and murine forms are only approximately 60% identical. For example, by contrast, the murine and human insulin receptor precursors are 95% identical when comparing the entire 1370 residues sequences. The selection pressure driving rapid divergence of the TNF receptor family members may well arise from the subversion of these systems by pathogens. Thus, a number of TNFR viral ORFs (SFV-T2, va53, SalF 19R, MYX-T2, G4R, and crmB) have been detected by sequence homology and found to be soluble TNF binding proteins capable of blocking TNF action.[20,22,69] These viral genes were presumably acquired from a host species by recombination and confer a selective advantage for the viruses by attenuating host immune and inflammatory responses. As a corollary of this argument, one would expect that evolution of a mutated ligand-receptor pair that was no longer inhibited by the viral gene product would be advantageous to the host.

Comparison of the cytoplasmic sequences of the receptors shows these to be considerably more diverse than the extracellular regions. Indeed, these differ markedly not only in sequence but also in size (Tables 2 and 3), and multiple sequence alignments show no evidence of any underlying shared structure running through the family. One striking comparison is that between murine and human CD40; whereas the membrane proximal 34 residues are 78% identical, the murine molecule possesses an additional 27 residues

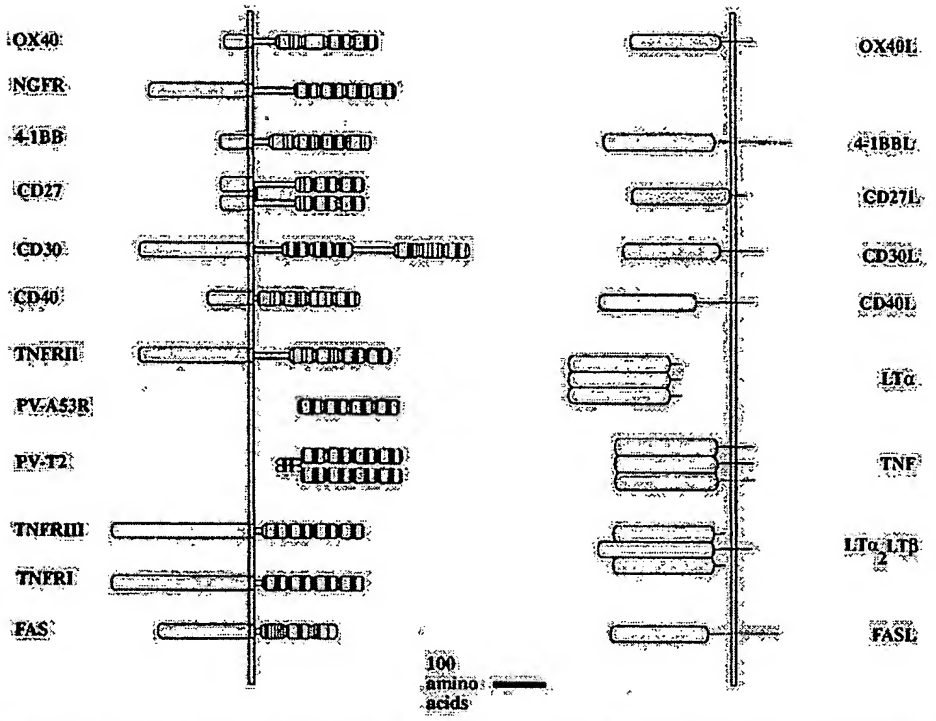


Fig 1. The TNF receptor and ligand superfamilies. Schematic presentation of the 10 members of the TNF receptor superfamily and two examples of viral ORFs (PV-A53R and PV-T2) encoding soluble TNFR homologues (left panel). The TNF receptor family members are characterized by variable numbers of cysteine-rich repeats in the extracellular domain. The homologous domains are shown as sequested open boxes, with the cysteine residues indicated by lines. TNFRIII is identical to the TNFR-RP (related protein). In addition, the nine members of the TNF ligand superfamily are shown in the right panel. The extracellular homologous C-terminal regions are indicated by open boxes and the nonhomologous sequences by lines. LT is shown in both the homotrimeric, secreted LTα form and the heterotrimeric, membrane-anchored LTα$_2$LTβ complex. Pro-TNF and FASL can be proteolytically cleaved for the release of active soluble forms. NGF is a basic, soluble dimeric molecule and the prototype of the NGF ligand superfamily. NGF has no homology to the TNF ligands and is therefore not included.

at the C-terminus. There are some elements shared between subsets of family members, thus TNFRI and FAS share the so-called "death domain",[78-80] but this sequence element is missing in the other two TNF receptors (TNFRII and TNFRP/TNFRIII).[10,12,77] Whether this degree of diversity is the consequence of rapid accumulations of mutations in this region of the genes for these molecules or of recombination events is not clear. However, it seems reasonable to suppose that there is unlikely to be any common immediate-early signalling event triggered through all these receptors. Indeed comparison of the sequences of this group of molecules as a whole with the databases fails to identify any motifs that resemble other families of signalling molecules, thus offering no clues as to what the immediate early events for any of these molecules might be. Relatively little general information is yet available about the signal pathways to which the more recently characterized family members (CD40, CD30, 4-1BB, CD27, OX40, and FAS) might couple, but a diverse set of signalling pathways seems likely for the divergent biologic responses. Most data for signalling through TNF receptors have come from studies using the TNFRI (p60) and TNFRII (p80) system and have been reviewed in detail recently.[81]

The extracellular regions of TNF ligand family, like the receptors, are highly diverse in sequence, with identity levels in the region of 20% for different family members. The murine and human forms of the same molecule are somewhat more conserved than the corresponding receptors, being approximately 80% identical (Table 3C). However, sequence alignments show that as with the receptors there is a characteristic pattern of sequence conservation, there being 9 short regions of conserved sequence distributed along the length of the molecules (Fig 2). Superposition of the sequences on the three-dimensional structures of TNF and LTα shows that these regions correspond to the strands that form the core of the protein. The residues that lie on the loops joining the strands show no detectable conservation. Thus the ligand family has diverged as rapidly as the receptors. Several of the ligand superfamily members have moderate sized cytoplasmic regions, and at least some are capable, when engaged by their receptors, of delivering signals (eg, CD27L, CD40L, and 4-1BBL).[82-84] The signal pathway is presently unknown.

CHARACTERISTIC BIOLOGIC PROPERTIES OF TNF LIGAND SUPERFAMILY MEMBERS

TNF and LTα, products of activated macrophages and T cells kill some transformed cell lines, mediate cell activation and proliferation, and are functionally linked as primary mediators of immune regulation and inflammatory response.[85,86] TNF has a pathogenic involvement, eg, in septic

Table 1. Molecular Characteristics for the Human TNF Receptor and Ligand Superfamily Members

Molecule	Molecular Weight (kD)	Length (amino acids)	Chromosomal Location
TNFR family			
CD27	45-55	242	12p13
CD30	120	578	1p36
CD40	50	258	20q11-13
4.1BB*	35	234	1p36
OX40	50	250	1p36
FAS/APO-1	45	320	10q24.1
TNFRI (p60)	60	435	12p13
TNFRII (p80)	80	440	1p36
TNFRIII (TNFR-RP)	~75	414	12p13
NGFR p75	75	402	17q21-22
TNFL family			
CD27L	50	193	19p13.3
CD30L	26-40	234	9q33
CD40L	33	261	Xq26-27
4.1.BBL*	50	309	19p13.3
OX40L	26-28	183	1q25
FASL	40	281	1q25
TNF	17 (s) and 26 (m)†	233	6 (MHC)‡
LTα	25	205	6 (MHC)
LTβ	33	244	6 (MHC)

* 4-1BB and 4-1BBL represent the murine proteins.

† Soluble (s) and membrane-bound (m) forms of TNF.

‡ TNF, LTα, and LTβ chromosomal mapping as a cluster within the location for the major histocompatibility complex (MHC).

shock, some autoimmune disorders, malignancies, and graft-versus-host disease.[8]

The nine TNF-related cytokines show distinctive but overlapping cellular responses for developmental and regulatory networks involving cells of the lymphoid, hematopoietic, and other lineages, such as stromal cells and neuronal cells.[4,5] At least some of the nine TNF ligand superfamily members (eg, TNF, LTα, LTβ, and CD40L) form trimeric proteins (see above).[35,71,72,87-89] In general, the TNF ligand superfamily members exert their biologic activity by causing receptor multimerization at the cell surface.[73] As mentioned above, the LTα homotrimer is the only entirely secreted protein and the TNF homotrimer is active after proteolytic release form the cell surface.[29,51,52] The biologically relevant forms of the other family members are membrane-bound type II glycoproteins.[35-38,43,45-47,90] Natural soluble forms for CD27L, CD30L, CD40L, 4-1BBL, and OX40L have not been reported. The exception is FASL, which exists in the predicted membrane-bound form but also as a soluble shed form in COS cell supernatants with presently unknown biologic relevance.[47]

Most of the TNF receptor superfamily members exist also in a soluble form, released by proteolytic cleveage (eg, TNFR p60, TNFRp80, CD27, CD30, CD40, and FAS) or through alternative splicing (eg, 4-1BB).[43,91-103] Although the cytoplasmic domains of most TNF receptor superfamily members are divergent from each other, several biologic functions, such as cytotoxic signals, induction of proliferation and differentiation, and cellular activation, are shared between two or more ligands.[5] Biologic activities related to T-cell–mediated immunity are a unique feature for all members of the TNF ligand superfamily.[8,35-37,43,45,47,104] All ligands and receptors, without exception, are expressed on activated T cells (Table 4). Purified human T cells and T-cell clones show enhanced proliferation when stimulated with any recombinant TNF family ligand or crosslinked with antireceptor antibodies in the presence of anti-CD3 or other mitogens, such as phytohemagglutinin (PHA), phorbol myristate acetate (PMA), or ionomycin.[8,35-37,43,45,47,104] Possible autocrine T-cell activation and growth control might be a common feature of this protein family. The induction of each ligand expression shows unique kinetics consistent with different roles for each of these ligands in the T-cell activation.[105] For example, the induction of CD30L surface expression on activated T cells is slower in comparison to other TNF ligands such as TNF, CD27L, CD40L, and 4-1BBL (maximal expression, 24 hours *v* 6 hours, respectively). B-cell proliferation and Ig secretion is induced by at least TNF, LTα, and CD40L. Further, several members participate in T-cell–dependent help for B cells, which are known to express TNFR-I, TNFR-II, CD27, CD30, CD40, FAS, and 4-1BB (Table 4).[106,107] TNF, LTα, and CD40L are mitogenic to B cells.[38,108-110] TNF, CD30L, and 4-1BBL are also abundantly expressed by activated macrophages.[30,37,43,84] Signals generated by TNF superfamily ligands in target cells are productively coordinated with accessory molecule expression (eg, LFA-1, ICAM-1, and B7 ligands).[111,112] For example, TNF, LTα, CD30L, and CD40L are capable of inducing cellular aggregation and upregulation of LFA-1 (CD11a)/ICAM-1

Table 2. Structural Characteristics of TNF Receptor and Ligand Superfamily

	No. of Amino Acid Residues			
	Extracellular	Transmembrane	Cytoplasmic	Extracellular Domains
Receptors				
CD27	175	21	46	2.5
CD30	360	27	211	5.5
CD40	175	21	62	4
FAS	156	20	144	3
OX40	188	26	36	3.5
TNFRI	190	25	220	4
TNFRII	240	27	173	4
TNFRIII	201	26	187	4
4-1BB	159	30	45	3.5
NGFR	225	23	154	4
Ligands				
CD27L	165	16	12	
CD30L	172	26	36	
CD40L	216	23	22	
LTα	170	24	11	
LTβ	197	31	16	
OX40L	139	21	23	
TNF	176	28	29	
4-1BBL	206	21	82	
FASL	179	27	75	

With the exception of 4-1BB and 4-1BBL, all sequences used in the analysis were those of human proteins.

Table 3. Sequence Relationships Between Members of the TNF Receptor Superfamily and Between Members of the TNF Ligand Superfamily

A. Receptor Extracellular Regions

	MuOX40	HuNGFR	Mu4-1BB	HuCD27	HuCD30a	HuCD30b	HuCD40	MuCD40	HuTRII	HuTR-RP	HuTRI	HuFAS
HuOX40	63.8	37.5	27.0	28.0	24.2	20.7	33.0	30.2	35.3	28.0	20.9	26.2
MuOX40	—	31.6	26.3	29.3	26.5	18.9	32.0	30.3	34.3	32.1	26.8	26.7
HuNGFR	—	—	25.3	25.8	25.6	25.8	25.6	28.4	26.4	26.3	34.4	23.3
Mu4-1BB	—	—	—	26.4	16.8	16.9	30.8	32.6	31.7	29.5	17.3	27.5
HuCD27	—	—	—	—	19.5	22.5	22.8	25.7	25.5	23.3	21.3	25.7
HuCD30a	—	—	—	—	—	50.9	24.2	25.2	28.7	29.3	29.1	23.1
HuCD30b	—	—	—	—	—	—	19.1	21.0	24.4	18.6	18.7	16.0
HuCD40	—	—	—	—	—	—	—	59.3	37.0	35.3	26.1	31.1
MuCD40	—	—	—	—	—	—	—	—	32.7	30.1	27.2	35.3
HuTNFRII	—	—	—	—	—	—	—	—	—	32.0	23.3	25.2
HuTNFR-RP	—	—	—	—	—	—	—	—	—	—	27.0	33.8
HuTNFRI	—	—	—	—	—	—	—	—	—	—	—	28.9

B. Receptor Cytoplasmic Regions

	MuOX40	HuNGFR	Mu4-1BB	HuCD27	HuCD30	HuCD40	MuCD40	HuTRII	HuTR-RP	HuTRI	HuFAS
HuOX40	61.1	22.2	18.9	13.9	30.5	16.1	21.6	18.3	27.0	14.3	17.4
MuOX40	—	24.2	26.7	23.3	11.4	14.3	14.3	11.7	33.3	30.3	8.8
HuNGFR	—	—	18.2	19.6	18.8	27.6	16.9	21.4	15.5	20.7	15.2
Mu4-1BB	—	—	—	28.5	19.5	21.6	19.0	17.1	24.3	12.2	7.5
HuCD27	—	—	—	—	28.9	18.6	23.4	11.9	20.9	21.7	18.6
HuCD30	—	—	—	—	—	33.8	15.6	24.7	27.2	18.6	9.7
HuCD40	—	—	—	—	—	—	77.9	23.3	23.7	21.0	25.0
MuCD40	—	—	—	—	—	—	—	20.8	20.7	15.9	11.4
HuTNFRII	—	—	—	—	—	—	—	—	20.1	16.1	15.6
HuTNFR-RP	—	—	—	—	—	—	—	—	—	21.4	14.5
HuTNFRI	—	—	—	—	—	—	—	—	—	—	23.9

C. Ligand Extracellular Regions

	Mu4-1BBL	HuCD27L	HuCD30L	HuCD40L	MuCD40L	HuTNF	HuLTα	HuLTβ	HuFASL	MuFASL
HuOX40L	13.6	17.7	20.9	20.3	19.3	15.5	20.0	17.8	23.5	22.1
Mu4-1BBL	—	22.6	23.8	20.1	20.2	23.2	19.9	23.4	23.1	24.5
HuCD27L	—	—	20.7	27.9	20.9	23.1	22.1	22.1	21.4	20.3
HuCD30L	—	—	—	23.0	21.3	25.5	16.3	20.4	22.1	24.7
HuCD40L	—	—	—	—	75.2	26.0	21.7	24.4	24.9	24.6
MuCD40L	—	—	—	—	—	30.1	21.7	23.1	23.7	24.0
HuTNF	—	—	—	—	—	—	33.5	26.0	26.4	31.7
HuLTα	—	—	—	—	—	—	—	30.9	29.6	28.2
HuLTβ	—	—	—	—	—	—	—	—	26.4	31.7
HuFASL	—	—	—	—	—	—	—	—	—	80.7

The sequences were compared in individual pairs using BESTFIT. For the receptors, the regions compared were those defined in Table 1. For the ligands, the regions compared were the entire region C-terminal to the membrane spanning sequence. The values given are percent amino acid identity, allowing gaps to be placed to optimize the fit. The extracellular region of CD30 contains the same concensus sequences as the other receptors, but has been duplicated (see Fig 1) to be able to compare it directly with the other members; each of the duplicated regions was treated separately. CD30a is residues 23-190 of CD30 (numbered from the initiator Met) and CD30b is 191-379.

Abbreviations: Hu, human; Mu, murine; TRII, TNFR II (p80); TRI, TNFR I (p60); TR-RP, TNFR-RP (related protein).

(CD54) expression.[113-117] CD30L and CD40L share the ability to induce B7-1 and B7-2 expression, part of the strongest known T-cell costimulatory pathway.[117-120] In general, all TNF ligand superfamily members, including FASL and CD40L, are essential for T-cell costimulation and activation. It is of special interest that signals, at least through CD27L, CD40L, and 4-1BBL, can provide costimulation for activated peripheral blood (PB) T cells.[82-84] Further studies need to be performed to see if other TNF ligand superfamily members are able to transduce a costimulatory signal.

The ability to induce cell death (necrosis and/or apoptosis) is another unique feature of this family and is presently established for TNF, LTα, CD30L, 4-1BBL, and FASL.[30,33,37,44,47,121,122] FAS and TNFRs expression is found broadly on both myeloid, lymphoid, and stromal cells.[10,123-125] FAS monoclonal antibodies (MoAbs) and FASL induce apoptotic (programmed) cell death and FAS/FASL interaction appears to be involved in T-cell repertoire formation, including positive or negative selection, suggesting a role of the FAS-FASL interaction in peripheral T-cell tolerance.[47,126-136] Interestingly, the cytoplasmic domains of the 60-kD TNFR and the FAS antigen contains a 65 amino acid "death domain," which is critical for signal transduction of the cytotoxic effects.[78,137] Both receptors still use at least partially distinct signaling path-

ways involved in apoptosis.[80] The cytoplasmic domains of the p60 and p80 TNFRs are unrelated and the signaling of the p80 TNFR for cell death and in mediating TNF responses in general remains controversial.[78,79,93,138-147]

Essential roles of several members of the TNF receptor or ligand family have been confirmed by naturally occuring or induced mutants that abolish the functional expression of the individual receptor/ligand protein. Naturally occuring inactivating mutations of the FAS antigen (lpr mouse) and the FASL (gld mouse) cause both similar lymphoproliferative diseases with lymphadenopathy and autoimmune disease, suggesting a failure of the immune system to eliminate autoreactive T cells.[49,50,148] CD40 and CD40L knock-out mice confirm that mutations of CD40L cause X-linked immunodeficiency, with high levels of IgM and low levels of IgG (block for Ig isotype switching).[149-151] Hyper-IgM patients show normal numbers and biologic function of B cells, but failure of T-cell–dependent B cell help because of nonfunctional CD40L.[39,152-155] Experimental deletion of the 60-kD TNFR gene in mice causes immunodeficiency with severely impaired clearance of bacterial pathogens and rapid death caused by infection, but resistance to the lethal effect of lipopolysaccharides (LPS).[156,157] Lack of 80-kD TNFR showed only a minimal phenotype with modest resistance to the lethal effect of TNF. In addition, functional ablation of TNF and LTα by overexpression of a neutralizing TNF inhibitory fusion protein (60-kD TNFR extracellular domain fused to mouse IgG heavy chain) in mice show pronounced LPS and TNF resistance with comparable phenotypic effect seen for the homozygous deletion of the 60-kD TNFR gene.[158,159] Furthermore, the deletion of the LTα gene results in a distinctive phenotype, characterized by the absence of structured lymph nodes and disordered splenic architecture.[160] In summary, several members of the TNF ligand and receptor superfamilies play crucial roles for lymphoid and thymic development, T-cell–mediated immune responses, T-cell–dependent help for B cells, and humoral B-cell activity. The detailed interactive network for the immune response and lymphoid differentiation mediated by the TNF-like ligands needs further evaluation.

CD27L AND CD70 ARE IDENTICAL MOLECULES AND ARE EXPRESSED ON DIFFERENT LYMPHOMAS

The CD70 antigen was originally identified by the Ki-24 MoAb.[161] CD70 is expressed on many peripheral T- and B-cell lymphomas (50% to 70% of cases positive) with frequent CD70 positivity observed within the cytoplasm.[162] The strongest expression is found on H-RS cells of HD (96% to 100% of cases positive) followed by large-cell NHLs (60% to 80% of cases positive). CD70 expression was not found on lymphoma cells derived from precursor T and B cells, such as lymphoblastic lymphoma or acute lymphoblastic leukemia (ALL). Permanent cell lines showed high CD70 expression only in those cell lines related to activated cells (eg, antigens, mitogens, and viral-transformed cells), but not in those resembling precursor T or B cells. In most cases, expression of the CD70 molecule is associated with the expression of other activation antigens, particularly CD25 and CD30.[162] However, 20% of B-cell NHLs and 5% of T-cell NHLs expressed only CD70 antigen on the lymphoma cells.[162]

For the immune system, CD70 is absent from resting lymphocytes, but can be induced after activation.[162] CD70 antigen is detectable on PHA-stimulated T and B lymphocytes after 24 hours and peaked at 96 hours, with 70% of stimulated peripheral blood B cells and 25% of T cells expressing the antigen.[162] CD70 expression could not be detected on resting or IFN-γ–treated monocytes, neutrophils, or dendritic cells. Recently, the CD70 gene was cloned and found to be identical to the cloned ligand for CD27 (CD27L).[36,83,90] CD27 is expressed by medullary thymocytes, most peripheral blood T cells, a subset of mature B cells, and NK cells.[163-170] CD27 expression on T cells is associated with the helper phenotype (naive T cells with CD45RA$^+$), whereas most memory T cells (CD45RA$^-$,CD45RO$^+$) lack CD27.[98,165,171] Activation of T cells results in upregulation of CD27 expression as cell surface molecule but also in the release of a soluble 28- to 32-kD form of CD27 (sCD27).[98-101,165,171] The proteolytic shed sCD27 molecule can serve as a marker for the immune activation in vivo.[13,172] The distribution and regulation pattern of CD27 for T cells supports a CD27 function for more naive/unprimed T cells than completely differentiated effector T cells.[98,165,171]

The biologic functions of CD27L include a costimulatory signal for T-cell proliferation, generation of cytotoxic T cells, and enhanced cytokine secretion,[36] but its functional relevance for thymocytes and B cells remains to be elucidated. It is of interest that only CD27$^+$ B cells can be induced to secrete Ig in vitro after stimulation with mitogens or CD27L.[164,165,172] In addition, CD27L antibodies or sCD27 block allogenic B-cell–mediated stimulation of T-cell proliferation.[90,164,165,172,173] Immunohistologic studies show that the CD27L/CD70 molecule is expressed on most lymphocytes in occassional tonsil germinal centers, low number of lymphocytes in the paracortical areas of tonsils, lymphoblasts in the skin and gut, and thymic epithelial cells, but not on cortical and medullary thymocytes.[162,171] Preliminary functional data suggest that the CD27-CD27L/CD70 interaction is part of the network involved in regulation of T-cell activation during antigen-specific immune responses, generation of memory T-cell populations, and expansion of cytotoxic T cells.[174]

A relatively high percentage of T- and B-cell NHLs but also HD express CD27L/CD70; this expression is characteristically high on H-RS cells.[162,175] In addition, most HD-derived cell lines express CD27L surface molecules but not the counterstructure CD27[63] (H.J.G., unpublished observation). B-cell NHL cell lines can express both the CD27 and CD27L/CD70 molecule, but its functional relevance for the growth control of these cells has not been established. CD27 expression was found in 50% of B-cell leukemias and 71% of B-cell NHLs.[176] CD27 was present on malignant B cells corresponding to early stages of antigen-independent B-cell maturation. Pro-B–cell ALLs were CD27$^-$, but 30% of pre-B–cell ALLs were positive. Mature B-cell ALLs had a high level of expression of CD27 and chronic lymphocytic leukemia (CLL), prolymphocytic leukemias, and some hairy cell leukemias (HCL) were moderate to strong CD27$^+$. In addi-

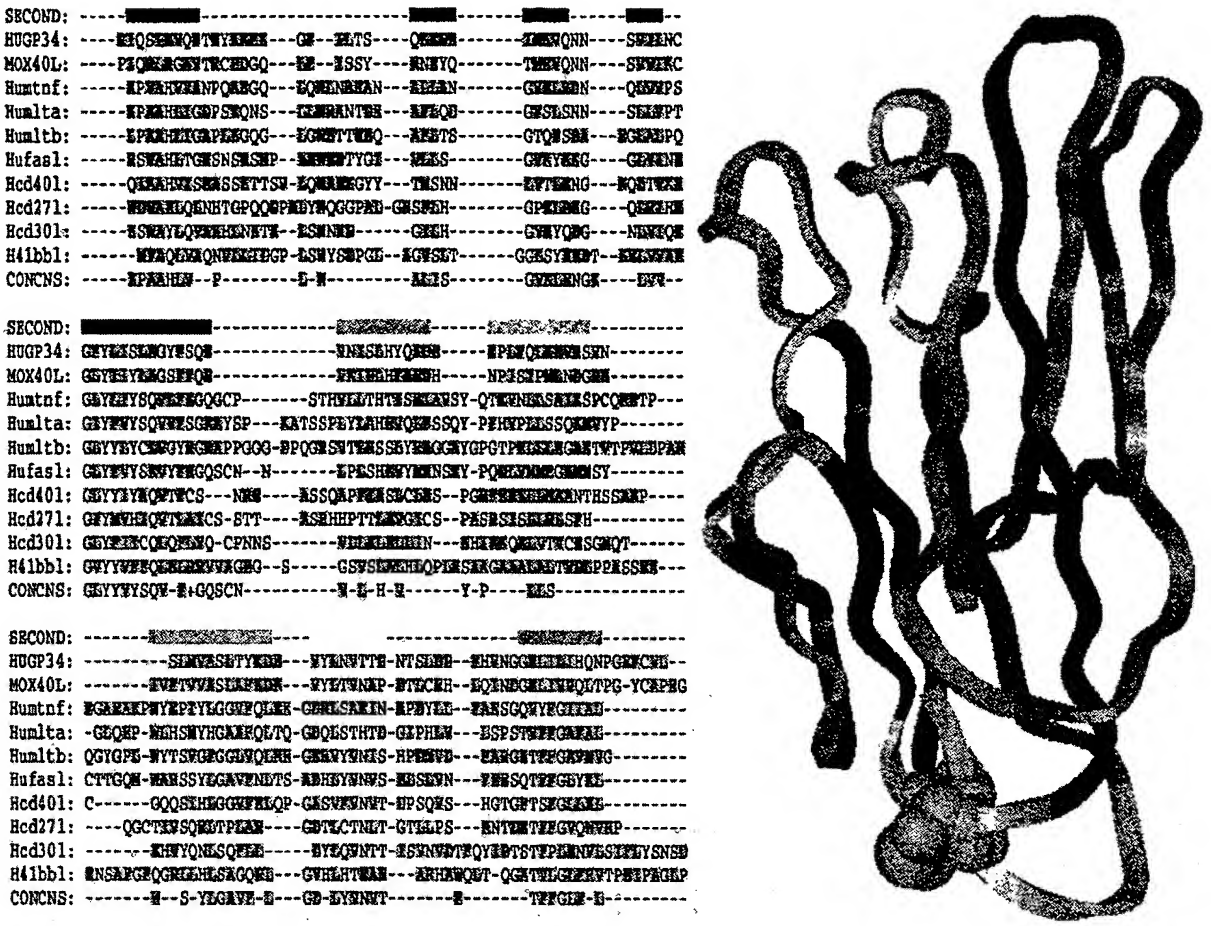


Fig 2. TNF ligand superfamily alignment and predicted tertiary structure based on TNF. Sequence homology is restricted to the C-terminal region of TNF family members (left panel). Colored bars on the top line (SECOND) indicate β-strands of TNF tertiary structure. Conserved amino acid motifs are shown by the line CONCNS. Amino acids are color-coded: green, hydrophobic; blue, basic; red, acidic; yellow, cysteine. (Right panel) Crystal structure of TNF with β-strands colored according to the corresponding sequence homology. Gray strands indicate nonhomologous amino acid. Hu and H, human; m, murine; l, ligand; tnf, tumor necrosis factor; lt, lymphotoxin; cd, CD (cluster designation).

tion, most low-grade diffuse and follicular lymphomas (85% of cases positive) and intermediate- and high-grade lymphomas (62% of cases positive with variable expression levels) expressed CD27 on the malignant B-cell population. Myeloma cells lacked expression of CD27. Furthermore, sCD27 was elevated in the serum of patients with B-cell malignancies and the highest levels were observed in patients with CLL and low-grade NHLs. The sCD27 serum levels showed a strong correlation to the tumor load, indicating sCD27 serum levels as a useful disease marker in patients with B-cell malignancies.[176]

Taken together, CD27 and CD27L/CD70 seem to be expressed with high frequency by the malignant cells of different entities of lymphomas and may serve as markers for tumor burden and disease activity, but any functional correlation to defined pathophysiologic presentation has not been established (Fig 3).

CD30 AN HD-ASSOCIATED ANTIGEN AND ITS LIGAND

HD-derived cell lines were used to develop MoAbs that could be used to visualize H-RS cells in tissue sections. Ki-1, the first CD30 MoAb, was raised against the HD-derived cell line L-428 and described to react uniquely with primary and cultured H-RS cells.[177] A small lymphoid cell population in reactive tonsils was also stained and postulated to be the precursor cells for H-RS cells.[178] Subsequent studies clearly showed that the CD30 MoAb (Ki-1) was neither cell-lineage restricted nor specific for H-RS cells.[179] Over the years, multiple MoAbs against the CD30 antigen (eg, Ber-H2, HeFi, HRS-1, HRS-2, HRS-3, M44, M67, and C10) have been generated with better immunochemical properties than Ki-1.[37,122,180-183] CD30 MoAbs detect a phosphorylated 120-kD membrane glycoprotein and its nonphosphorylated 84-kD precursor protein.[184,185] The cloning of the CD30 antigen has suggested that it might act as a cytokine receptor.[14,186]

In addition to the CD30 staining for a small cell population in the parafollicular area of hyperplastic lymph nodes and tonsils, most blasts appearing during infectious mononucleosis are positive for CD30 expression.[178,181,187,188] CD30 expression has also been detected on a subset of mitogen- or antigen-activated PBTs, Epstein-barr virus (EBV)-

Table 4. Role of the TNF Ligand Superfamily Members for T- and B-Cell Activation Involved in the Immune Response

Function	CD27L	CD30L	CD40L	4-1BBL	OX40L	FASL
Expressing cells	T*, B*, Mf	T*, Mf*, G	T*	T*, S, Mf*, B*†	T*†	T*
Responding cells	T*‡, B*, NK	T*, B†, NK	B, EP, Mf*, T*	T*, Mf	T*	T†, B, Mf, G, S
Signaling through ligand	+	?	+	+	?	?
T-cell costimulation	+	+	+	+	+	+
B-cell proliferation		+	+			
Enhanced cytokine secretion	+	+	+		+	+
Ig secretion		+	+			
Upregulation of cell surface antigen expression	+	+	+			+
Upregulation of costimulatory molecules	+	+	+			+
Aggregation/adhesion		+	+			+
Apoptosis/necrosis/AICD		+§	−‖	+		+

Abbreviations: B, B cells; BA, basophils; EN, endothelial cells; EP, epithelial cells; G, granulocytes; M, mast cells; Mf, monocytes/macrophages; NK, natural killer cells; S, stromal cells/fibroblasts; T, T cells; AICD, activation-induced cell death.

* Strong expression after activation.

† Induction of expression after viral transformation (eg, HTLV-1, HTLV-2, EBV, and HIV).

‡ CD27 expression is found mainly for naive > memory T cells, but FAS expression for memory > naive T-cell populations.

§ Cytolytic and cytostatic effect on LCAL-derived tumor cell lines.

‖ Rescue of germinal center B cells from undergoing spontaneous apoptosis.

transformed B cells, and human T-lymphotropic virus types I and II (HTLV-I and -II) infected lymphocytes or cell lines.[178,180-182,184,189,190] In addition, it has been shown that, among T-cell clones, CD30 is mainly expressed and released as sCD30 from $CD4^+$ and $CD8^+$ T-cell clones producing T_H-2 cytokines such as IL-4 and IL-5.[191-193] Activated tonsil B cells have been reported to be $CD30^+$.[178,181] Similarly, human NK cell clones express the CD30 surface protein.[194] Furthermore, the expression of CD30 was seen by late-stage differentiated PB macrophages.[195] These data are controversial because other groups could not detect CD30 protein or mRNA expression by activated monocytes or macrophages.[63,122,178,181]

CD30 protein and mRNA expression are found in all HD-derived cell lines, with the exception of the myelomonocytic cell line HD-MyZ and cell line SUP-HD1.[14,62,63,122,196] The CD30 antigen is seen on the majority of H-RS cells of most HD cases, with the exception of the lymphocyte-predominant (LP) subform.[61,63] Overall, 84% to 91% of the lymphocyte-depleted (LD), mixed cellularity (MC), and nodular sclerosing (NS) HD cases, but only 32% of LP HD cases express CD30 on the diagnostic primary H-RS cells.[177,178,181,197-199] CD30 expression is not restricted to the malignant cells of HD because CD30 MoAbs also identify a new entity of NHLs with anaplastic morphology ($CD30^+$ anaplastic large-cell lymphomas [ALCL]).[178] In addition to H-RS cells of HD and the subgroup of $CD30^+$ ALCLs, CD30 is also expressed to variable extents on several histologic subtypes of other NHL, such as cutaneous T-cell lymphoma, nodular small cleaved-cell lymphoma, lymphocytic lymphoma, peripheral T-cell lymphoma, Lennert's lymphoma, immunoblastic lymphoma, adult T-cell leukemia/lymphoma

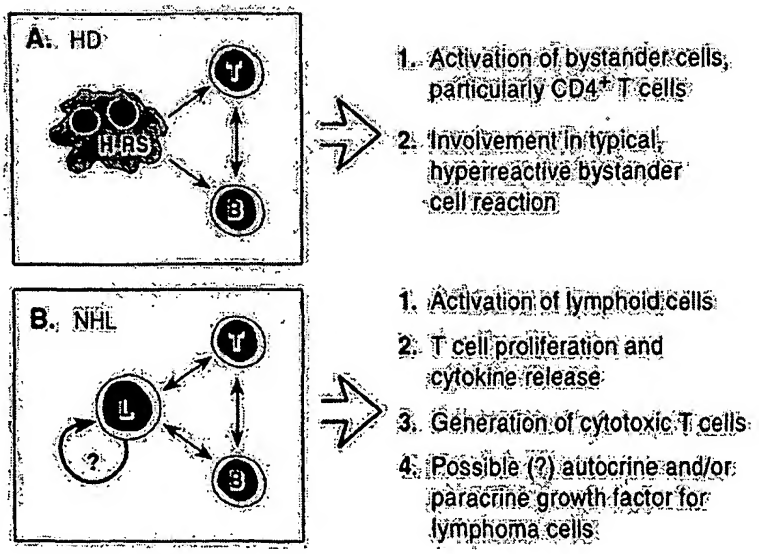


Fig 3. Expression and potential functional involvement of CD27L for HD and NHL. (A) For HD, CD27L (CD70) expression is found for most H-RS cells and surrounding B and T cells. CD27L might be critically involved in hyperreactive bystander cell reaction. (B) NHL cases show frequent coexpression of CD27 and CD27L. CD27L could be an autocrine and/or paracrine growth factor for lymphoma cells.

(ATLL), T-acute lymphoblastic leukemia (T-ALL), and centroblastic/centrocytic follicular lymphoma.[178,197,200-210] Taken together, around 10% of NHLs are positive for CD30 on their malignant lymphoma cells (32% of T-cell NHLs and 4% of B-cell NHLs).[63,186] CD30 expression in T-cell NHLs of different subtpes is restricted to the large-cell variants.[186] The functional relevance of CD30 expression for most entities of NHLs remains presently unclear. The association between CD30 expression and lymphoid malignancies has proven to be a useful pathologic and clinical marker for the identification of malignant cells within lymphoid tissues, particularly lymph nodes. However, expression of CD30 has also been reported on some embryonal carcinomas, nonembryonal carcinomas, malignant melanomas, mesenchymal tumors, some myeloid cell lines, and decidual cells.[181,198,211-213] The CD30 antigen is suitable for immuno-imaging using immunoscintigraphy with radioiodine-labeled antibodies[182] and immunotherapy using immunotoxins (ricin-A or saporin conjugated MoAbs) in HD patients.[186,214,215]

The 85-kD sCD30 molecule is detectable in the serum under restricted conditions.[92] Detectable high sCD30 serum levels were found in the majority of HD cases at diagnosis, more often in patients with advanced disease, bulky tumor, and/or presence of constitutional B symptoms.[216-219] Elevated sCD30 serum levels correlate with the clinical presentation, such as stage, presence of B symptoms, and tumor burden. The sCD30 in the serum of HD patients derives most likely from the CD30$^+$ H-RS cells and is associated with the extent of neoplastic infiltration in HD-involved areas.[177,178,217,218] Most cases with LD HD had positive serum levels, but LP HD patients were frequently sCD30$^-$.[220,221] Soluble CD30 was not detected in any HD patients in complete remission. The levels of sCD30 represent an independent prognostic factor with high sCD30 levels being associated with reduced disease-free survival.[219] The detection of sCD30 seems to be a useful "tumor marker" for CD30$^+$ HD patients based on its correlation to disease activity and presence of H-RS cells.

Recently, the ligand for the CD30 antigen has been cloned and biologically characterized.[37] The presence of the CD30 counterstructure (CD30L) confirms the presumed cytokine receptor function of CD30. The CD30L is a 26- to 40-kD type II membrane glycoprotein, mainly expressed on activated T cells and monocytes/macrophages but also on granulocytes and some Burkitt's lymphoma cell lines.[37,122,222]

Nonpathologic, CD30 expression is largely restricted to antigen-activated T cells and is not detectable before complete and functional antigen receptor rearrangements.[105,178,180,181,184,189,190] IL-2 is further able to enhance mitogen- or antigen-induced CD30 expression on CD45RO$^+$ PBT cells.[190] In contrast, CD30L expression is broader and has been found on CD4$^+$ and CD8$^+$ activated T cells with all CD45 isoforms.[37,105] CD30L costimulates T-cell proliferation[37] and enhances induction of activation antigens, such as ICAM-1, on activated T cells.[105] In addition, CD30L upregulates cytokine secretion of activated PBTs such as IL-2, IFN-γ, and TNF but not IL-4.[105] CD30$^+$ T-cell clones (CD4$^+$ and CD8$^+$) produce preferentially T_H-2 cytokines (eg, IL-4 and IL-5), but peripheral blood T cells stimulated through CD30 release mainly T_H-1 cytokines (eg, IL-2 and IFN-γ).[105,191,192,223] Further studies are needed to determine whether the heterogenous population of CD30$^+$ T cells contains T cells that are capable of developing into both T_H-1 and T_H-2 phenotypes or whether clonal CD30$^+$ T cells are exclusively from the T_H-2 subtype. In general, CD30L is a part of the cascade involved in antigen-induced T-cell activation and proliferation and might play a pathogenic role in several immunologic diseases associated with T_H-2 cytokine pattern (eg, systemic lupus erythematosus, atopic disorders, Omenn's syndrome, and human immunodeficiency virus [HIV] infection).[193]

The analysis of 105 continous human leukemia-lymphoma cell lines for CD30L showed a restricted expression pattern for 6 of the 26 B-cell–lineage tumor cell lines (4 CD30$^-$ Burkitt lymphoma [BL] cell lines, 1 CD30$^-$ BL-like ALL, and 1 NHL).[222] All HD-derived cell lines were CD30L mRNA and surface protein expression negative.[122] Recombinant CD30L was mitogenic for the "T-cell–like" HD-derived cell lines HDLM-2 and L-540 and the T-ALL cell line KE-37 but not for the "B-cell–like" HD-derived cell lines KM-H2 and L-428 or CD30$^+$ BL cell lines.[122] In addition CD30L is capable of enhancing IL-6, TNF, and LTα secretion for HD-derived cell lines and upregulates surface expression of ICAM-1 and B7 family members in a similar fashion as seen for CD40L (see below).[117] CD30L seems to be another paracrine-acting molecule involved in the deregulated cytokine and activation cascade, characteristic for HD.[64] CD30L could be produced by H-RS cell surrounding bystander cells, such as activated CD4$^+$ T cells and activated macrophages and granulocytes (Fig 4). CD30L could modify cytokine secretion of H-RS cells and enhance proliferation and activation of H-RS cells, including upregulation of cell contact-dependent signals. The overexpression of the cytokine receptor CD30 on most H-RS cells appears to be an important clinical, biologic, and pathologic marker for HD (Fig 4). Further understanding of the CD30-CD30L interaction for the oncogenesis of HD will be hopefully generated from studies analyzing CD30L expression in primary HD cases and in vivo models, such as the HD-SCID mice system.

CD30L TRANSDUCES ANTIPROLIFERATIVE SIGNALS TO CD30$^+$ ALCLs

CD30$^+$ ALCLs (approximately 10% of all NHLs) express characteristically high amounts of CD30 on the surface of their clustered malignant lymphoma cells with either T, B, or O phenotype.[178] CD30$^+$ ALCLs are characterized by the presence of large, pleomorphic tumor cells; expression of lymphocyte activation antigens (eg, CD25, CD30, CD71, and MHC class II); a frequent nonrandom chromosomal abnormality (t:2;5); and frequent extranodal disease affecting the skin, lung, gastrointestinal tract, soft tissue, and bone.[178,224-230] CD30$^+$ ALCL can be confused with HD because of the presence of occasional H-RS–like cells and the overlapping immunophenotype of the tumor cells.[178,199,224-227,231] In practice, H-RS cells in HD are frequently CD15$^+$, CD30$^+$, CD45$^-$, EMA$^-$, in contrast to ALCL tumor cells with CD15$^-$, CD30$^+$, CD45$^+$, EMA$^+$ immunophenotype.[232,233] HD is in 50% to 60% of cases associated with EBV.[63] The association of EBV with CD30$^+$ ALCLs remains controver-

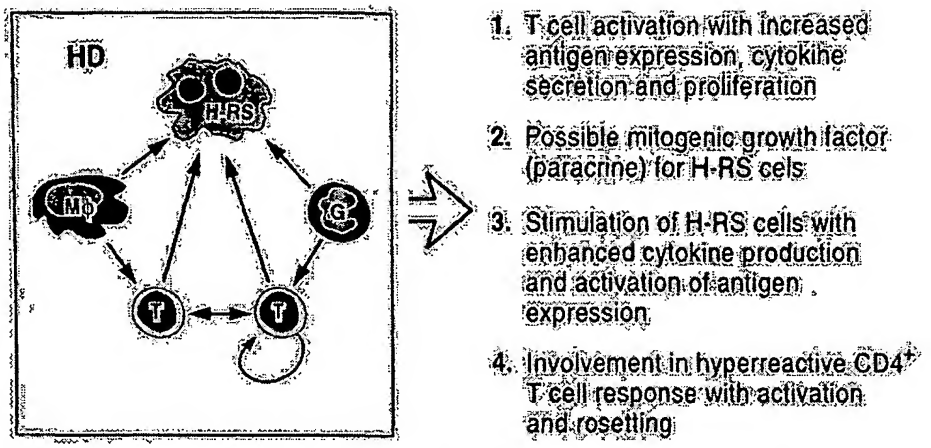


Fig 4. Schematic presentation for the possible functional role of CD30L for the pathology of HD. CD30L expression is found as membrane-bound protein on activated T cells, monocytes/macrophages, and granulocytes (cell types all involved in hyperreactive bystander cell reaction). H-RS cells are CD30L−. CD30, as an HD-associated antigen, is expressed by most primary H-RS cells (lymphocyte-predominant subform is an exception). The CD30-CD30L interaction between CD30+ H-RS cells and CD30L+ bystander cells could be part of the deregulated cell-cell interaction, including growth stimulation, upregulation of activation antigens, and enhancement of cytokine release.

sial (range, 0% to 67% of cases positive), but most data would support the lack of a strong relationship between CD30+ ALCLs and EBV.[234-237] These findings suggest that the presence of CD30 is not a simple relation to EBV infection in malignant lymphomas.

Several EBV− ALCL-derived cell lines show strong CD30 expression and could be biologic targets for CD30L. In contrast to the HD-derived cell lines, CD30 mediates reduction of proliferation and cell growth arrest (antiproliferative effect) for most of the CD30+ ALCL cell lines.[122] The antiproliferative effect of CD30L includes a cytolytic effect and a cytostatic component with cell cycle arrest.[122] The mechanism for the cytolytic effect is presently unlear, but seems to be FAS-independent and not associated with apoptotic DNA fragmentation. Further investigations have to clarify the interaction between tumor growth of the CD30+ anaplastic lymphoma cells, tumor progression/regression, and CD30L expression in ALCL-involved tissues (Fig 5).

It is of interest that the t(2;5) translocation is a common chromosomal finding for CD30+ ALCLs.[228,230] This rearrangement fuses the nucleolar phosphoprotein nucleophosmin (NPM) gene on chromosome 5q35 to the novel tyrosine kinase gene ALK (anaplastic lymphoma kinase) on chromosome 2p23.[238] The association of the overexpression of the truncated ALK-fusion protein with the malignant transformation in the CD30+ ALCLs is presently unclear, but an 80-kD protein tyrosine kinase (identical to ALK) has been suggested to physically interact with CD30.[239] It is of interest that only a fraction of CD30+ ALCL (12% to 40%) cases show the rearrangement of the NPM gene and that at least

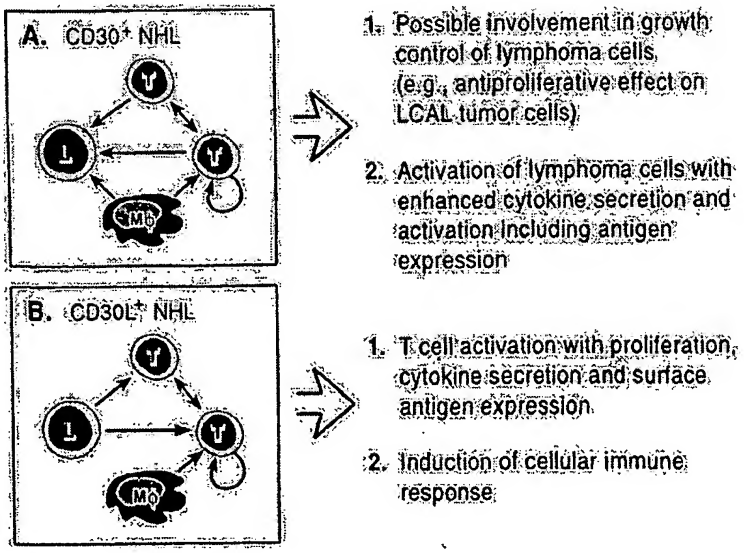


Fig 5. Pathologic role of CD30 and CD30L for NHLs. NHL cells (L) express either CD30 (A) or CD30L (B). CD30 expression of NHL cells could be involved in cellular activation, such as release of cytokines or enhanced expression of activation antigens, and in growth control. A subgroup of ALCL cells use CD30 to mediate an antiproliferative effect. The main source of CD30+ cells is activated T cells. CD30L+ lymphoma cells might induce T-cell activation and cellular immune responses.

a fraction of HD cases may contain the NPM/ALK fusion transcripts (3 studies using reverse transcriptase-polymerase chain reaction [RT-PCR]: 0 of 40, 2 of 9, and 9 of 13 HD cases rearranged).[240-242] Further studies have to show whether HD cases with rearranged NPM gene represent ALCLs that mimic HD or whether it presents a separate HD subform closely related to ALCL. In addition, CD30$^+$ ALCLs present frequently with abnormal c-myc gene products (6 of 18 cases [33%]), but the functional significance of this is unclear.[243] Taken together, recently several molecular alterations for CD30$^+$ ALCLs have been identified. Further studies will hopefully connect these molecular abnormalities with the functional role of CD30-CD30L interaction for oncogenesis and pathogenesis of ALCLs. In addition, the prognostic, biologic, and functional role of CD30 expression of the malignant cells of other NHLs needs further evaluation.

CD40L SHARES COMMON BIOLOGIC ACTIVITIES WITH CD30L FOR HD

CD40 is a 50-kD glycoprotein and is expressed on a variety of cell types, including normal, virally transformed and malignant B cells (see below for more details), but also monocytes, activated T cells, follicular dentritic cells, interdigitating reticulum cells, thymic epithelium, and some epithelial carcinomas.[15,162,244-246] Recently, the murine and human CD40L have been characterized and cloned.[38-42] The CD40L is a 33- to 39-kD type II membrane glycoprotein and is expressed primarily on the surface of activated CD4$^+$ T cells but also on some CD8$^+$ T cells, mast and stromal cell lines, and basophils.[40,247,248] Studies using MoAbs to CD40 or recombinant CD40L have shown diverse biologic activities as a result of signaling through CD40.[65,249] These activities include the proliferation of B cells and induction of Ig secretion in the presence of other cytokines.[250] Furthermore, CD40L-CD40 interactions mediate rescue of germinal center centrocytes from apoptosis.[251,252] CD40 exposure of thymic epithelial cells in the presence of IFN-γ and IL-1 induced GM-CSF release.[246] Signals through CD40 upregulate the expression of LFA-1, B7 ligands, ICAM-1, and CD23 with involvement in both homotypic and heterotypic cell adhesion and costimulation.[113,115,116,118-120,253-255] In addition, primary PB monocytes express low amounts of CD40 and cytokines, such as GM-CSF, IL-3, or IFN-γ, upregulate this CD40 surface expression.[256] CD40L induces/enhances cytokine secretion of PB monocytes (eg, IL-6, IL-8, and TNF) and potent tumoricidal activity of monocytes.[256] Recently, it was shown that CD40L also costimulates T-cell proliferation and enhances expression of CD25 and CD40L itself.[104] Furthermore, CD40L can transduce signals by its own.[82] Taken together, CD40L may play a complex role in the immune response by functionally interacting with CD40 expressed on the surface of B cells, monocytes, T cells, and some epithelial cells.

A series of HD-derived cell lines (exception the myelomonocytic HD-MyZ cell line) express at the mRNA and protein level not only CD30, but also CD40.[122,196,257,258] On the other hand, these cell lines are negative for CD30L and CD40L mRNA and protein expression.[122,258] Expression of CD40 by H-RS cells has been initially described as an indication for a follicular dendritic cell origin of H-RS cells.[259] Recently, three studies have been reported on high level CD40 expression of primary H-RS cells.[257,258,260] A total number of 156 HD cases have been investigated and primary H-RS cells in 145 HD cases express CD40 (95% of cases positive). HD-involved tissues with all four histologic subtypes expressed abundant amounts of CD40 by H-RS cells independent of CD30 expression and irrespective of their antigenic immunophenotype. Further studies have to investigate the relationship between the expression of cytokines (eg, IL-4 and IFN-γ) and/or EBV proteins (eg, LMP-1) known to upregulate CD40 expression of H-RS cells and the deregulated, strong CD40 expression of H-RS cells. Primary H-RS cells did not express CD40L, but scattered lymphoid cells in disease-involved areas of HD were CD40L$^+$.[258] It is of interest that at least in part the in vitro rosetting of CD40L$^+$ (activated), CD4$^+$ T cells to cultured CD40$^+$ H-RS cells is mediated through the CD40/CD40L adhesion pathway.[257]

Table 5. CD30L and CD40L Share Common Biologic Activities on Cultured H-RS Cells

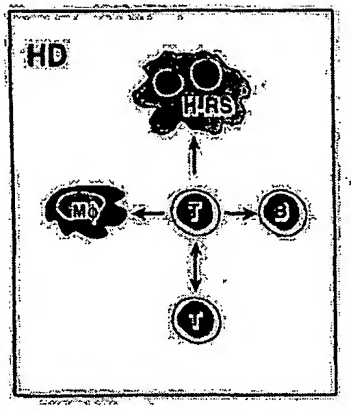
Function	CD30L	CD40L
Mitogenic activity	+	−
Enhanced clonogenic colony formation	+	+
Induction of cytokine secretion	+	+
Induction of ICAM-1 surface expression	+	+
Increased soluble ICAM-1 concentration	+	+
Aggregation/adhesion	+	+
Upregulation of costimulatory molecules (eg, B7 ligands)	+	+
Downmodulation of CD30 surface expression	+	+
Shedding of CD30	+	+
Shedding of CD40	+	+(?)

Functional analysis of the CD40$^+$ cultured H-RS cells showed a 50% increase of colony formation using a soft agar system,[257] but CD40L had no mitogenic activity on these CD40$^+$ HD-derived cell lines.[258] Recombinant CD40L induced IL-8 secretion and enhanced IL-6, TNF, and LTα from cultured H-RS cell lines.[258] In addition, CD40L enhanced the expression of activation and adhesion molecules, such as ICAM-1, B7-1, and B7-2, all of which are overexpressed on primary H-RS cells.[117,258,261-266] Furthermore, CD40L induced a 40% to 60% reduction of the expression of the HD-associated CD30 antigen with an increase of sCD30 levels.[258]

Taken together, CD30L and CD40L share pleiotropic biologic activities on H-RS cells such as induction/elevation of cytokine secretion and adhesion/activation surface molecule expression (Table 5 and Figs 4 and 6). The CD30-CD30L and CD40-CD40L interactions might be critical elements in the unbalanced cytokine network and cell contact-dependent activation cascade typical for HD.[64]

CD40 EXPRESSION AND MALIGNANT B-CELL NEOPLASIAS

CD40 is found on B cells at most stages of differentiation (with the exception of plasma cells), malignant B cells such as lymphomas and leukemias, and virally transformed B



1. Proliferation and activation of B cells, including Ig secretion
2. Induction of cytokine secretin and tumoricidal activity of monocytes
3. Costimulation of T cell proliferation and activation antigen expression
4. Upregulation of homo- and heterotypic cell adhesion and activation for normal lymphoid cells and H-RS cells
5. Part of the deregulated cytokine network present around H-RS cells

Fig 6. CD40 expression of H-RS cells. In addition to B cells, T cells, and monocytes, most H-RS cells express high levels of CD40 on their surface. CD40L expression is absent from H-RS cells, but is found with higher frequency on some surrounding bystander cells. CD40L might be involved in B-cell activation, costimulation of T cells, and stimulation of monocytes/macrophages, as well as in part of the typical features of HD such as cellular adhesion and deregulated cytokine secretion.

cells.[26,162,244-246,253,256,267,268] In general, CD40 surface expression is upregulated after activation, but downregulated on differentiation to plasma cells.[162] Antigen-specific activation of B cells requires a two-step signaling pathway with initial antigen binding, processing, and presentation with MHC class II on B cells, followed by recognition of the antigen by helper T cells with activation and expression of costimulatory signals for B cells (T-cell–dependent B-cell help).[106] Collaboration between antigen-presenting B cells and activated T cells is mediated both by soluble proteins (cytokines) and cell-cell contact-dependent membrane-bound (receptor-ligand for cytokines or activation antigens) interaction.[107] A combination of these signals directs a B-cell response with proliferation, antibody production, and isotype switching.

CD40 expression on B cells is of crucial importance for B-cell function.[269] Ligation of CD40 with MoAb induced proliferation of anti-IgM cross-linked or IL-4–stimulated B cells[244,253,270-272]; secretion of IgE, IgG, or IgM in the presence of various cytokines[255,273-276]; rescue of germinal center centrocytes from apoptosis[251,277]; activation of homotypic and heterotypic adhesion[113,115,253-255]; and induction of bcl-2 expression.[277] CD40 expression on B cells is enhanced by IL-4, IgM, CD20 MoAb, PMA, or IFN-γ.[15,26,270] A soluble form of CD40 has been detected in the supernatant of mitogen-activated primary B cells and EBV-transformed B-cell lines.[102]

The cloned CD40L is mainly expressed on activated $CD4^+$ T cells.[26,40,248] Induction of CD40L expression on activated T cells is rapid (maximum, 8 to 10 hours) and is tightly regulated (baseline level after 24 hours of activation).[40,248] As predicted, recombinant CD40L stimulates B-cell proliferation in the absence of costimuli and in combination with cytokines (eg, IL-4 for IgE and IgG_4; IL-2 and IL-10 for IgA, $IgG_{1,3}$, and IgM) stimulates secretion and/or isotype switching of B cells.[65,249] TGF-β inhibits CD40L-mediated secretion of Igs.[250]

CD40L gene defects have been identified as the causative factor for the inherited condition of a severe immunodeficiency known as X-linked hyper-IgM syndrome, characterized by elevated levels of serum IgM and low or nonexistent levels of IgG, IgA, and IgE; generalized failure to form germinal centers; and increased susceptability to opportunistic infection.[39,153-155,278-280] CD40L is a major pathway involved in T-cell–dependent help for B cells. Taken together, these studies confirm the essential role of CD40 for Ig heavy chain switching and the production of all Ig isotypes other than IgM.

Predicted on the pan-B-cell reactivity of CD40 for lymphoid organs,[162,244,245,253,267,268] several studies indicate that most or virtually all B-cell CLLs (80% to 90% of cases) and B-cell NHLs (90% to 100% of cases) express CD40.[162,244,245] In addition, 30% of biphenotypic or B-lineage ALLs and 90% of HCL express CD40.[245] Notably, the CD40 antigen is restricted to the B-lineage lymphoid cells, because none of the T-lineage ALLs, T-lineage CLLs, or T-lineage NHLs (exception ALCLs) stained with CD40 MoAbs.[245] In contrast, one study reported only 3 of 23 NHLs (12 B-cell NHLs and 11 T-cell NHLs) expressed CD40, including 2 B-cell NHLs and 1 T-cell lymphoblastic lymphoma.[260] These conflicting data may be explained by different sensitivities (affinity and/or off-rate) of the CD40 MoAbs used or the detection system for low (normal) level CD40 expression. CLL B cells and B cells from NHLs activated through CD40 show enhanced DNA synthesis after stimulation with B-cell trophic factors.[281] Of 29 $CD30^+$ ALCL cases investigated, 13 expressed CD40 (45% of cases), but CD40 expression was seen for cases with B-cell, T-cell, or null phenotype.[257,258] Large numbers of tumor cells were labeled and displayed moderate to strong staining.[257,258] These findings further support a pathologic association between $CD30^+$ ALCLs and HD, as suggested by the unique high-level expression of CD30, HD-lectin, IL-9, and c-kit for these two lymphoma entities.[178,282-284] The presence of the ALCL-associated translocation t(2;5) with rearrangement of the NPM gene in at least some HD cases further indicates a biologic relationship between ALCL and some HD cases, but the relationship for a common pathogenesis needs to be identified.[240-242]

Analysis of CD40L presence in vivo confirms restricted expression to small mononuclear cells in lymphoid tissue but not other tissues, such as muscle, brain, kidney, intestine, ovary, uterus, testes, skin, lung, or liver.[285] CD40L expres-

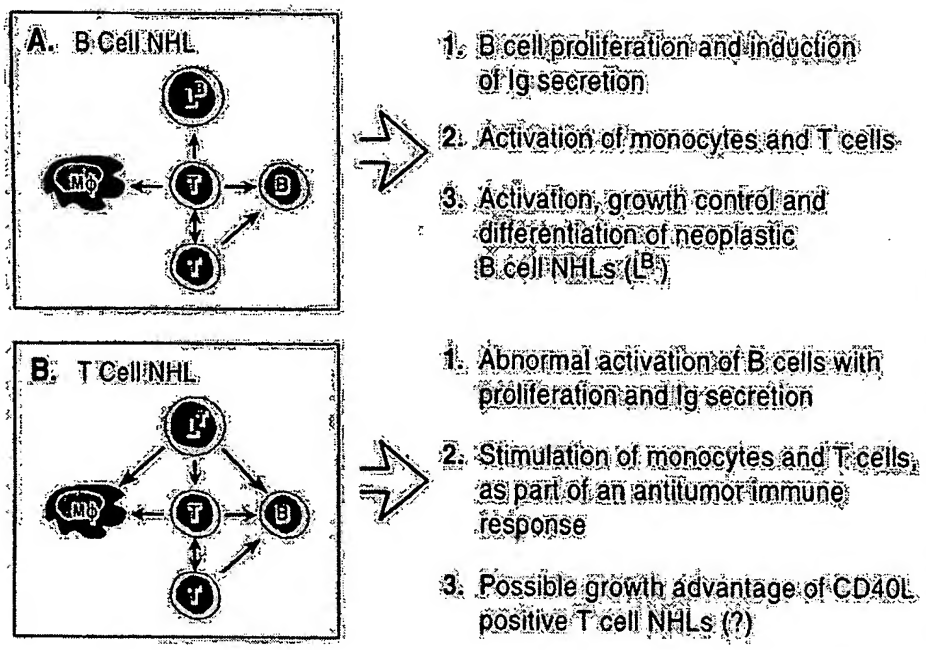


Fig 7. Schematic presentation of CD40-CD40L interaction for different NHLs. CD40 expression is found not only on B cells at most stages of differentiation and virally transformed B cells but also on most malignant B cells (L^B). (A) For CD40$^+$ B-cell NHLs, CD40L expressed by activated surrounding T cells might stimulate B cells, T cells, and monocytes/macrophages and, in addition, may also be involved in activation, growth control, and differentiation of L^B cells. On the other hand (B), CD40L$^+$ T-cell NHLs (L^T) might interact with surrounding CD40$^+$ immune cells and supporting cellular and humoral immune responses.

sion was preferentially localized in the mantle zone and germinal center light zone of secondary follicles of all peripheral lymphoid tissues and also the T-cell–rich periarteriolar areas in the spleen.[285] In vivo, CD40L-expressing cells are mainly CD4$^+$ T cells.[285] A panel of T-cell NHLs and leukemias (87 cases) was investigated for localization of CD40L expression.[286] Immunohistochemical staining showed that 21 of the 87 cases (24%) expressed CD40L with membrane and/or cytoplasmic immunoreactivity of a majority of neoplastic cells. In addition, the unexpected constitutive expression of CD40L by the neoplastic T cells was found mainly for CD4$^+$ tumors with a mature T-cell phenotype. The association of constitutive CD40L expression for some T-cell tumors with clinical and immunologic presentation such as hypergammaglobulinemia, monoclonal gammapathies, immune complex disease, or autoantibody syndromes needs to be established for the abnormal activation of B cells through malignant T cells. CD40L might provide a growth advantage for a subgroup of T-cell neoplasias.

An activation-induced growth arrest has been observed for several B- and T-cell malignancies through either antigen receptor or costimulatory receptors with antitumor effects when exposed to stimuli that lead to activation of the normal counterparts.[287-290] It is of great interest that anti-CD40 and soluble CD40 ligand significantly inhibited (40% to 60%) the in vitro proliferation of a series of B-cell lymphoma cell lines.[291,292] In addition, an antitumor effect of anti-CD40 treatment was observed in SCID mice bearing the human B-cell lymphoma lines with increased survival and inhibition of tumor growth.[291] Furthermore, anti-CD40 MoAb prevents human B-cell lymphomagenesis in the hu PBL-SCID mouse, but also promotes human B-cell engraftment in vivo.[293] CD40L may be of significant clinical use in new treatment modalities of CD40$^+$ NHLs with or without conjugation to immunotoxins or radioisotopes.

Taken together, CD40 expression on malignant B cells seems to be a frequent finding and a potential candidate for alternative treatment approaches, such as tumor targeting (Fig 7). Expression and the pathophysiologic role of CD40L for NHLs requires further functional analysis in vitro and in vivo (Fig 7).

ROLE OF FAS/FASL SYSTEM FOR LYMPHOMAS

Cell survival and function is controlled by proliferation and differentiation (positive selection) but also by cell death (negative selection).[294,295] The death of cells occurs by either programmed cell death with apoptosis or necrosis.[296,297] Apoptosis can be morphologically and biochemically distinguished from necrosis. In most cases, apoptosis is associated with condensation and segmentation of nuclei, loss of plasma membran microvilli, and degradation of chromosomal DNA into 200-bp nucleosome fragments.[295-297]

FAS and APO-1 antigens were identified by MoAbs with cytolytic activity against certain human cells and subsequently cDNA cloning.[18,19,134,135] FAS and APO-1 antigen are identical molecules and have been assigned to cluster CD95.[18,19,298] The CD95 antigen acts as a cell-surface receptor that is involved in apoptosis, including T- and B-cell deletion for the immune system.[47,126-134] In vivo, CD95 MoAbs induce rapid tumor regression.[134,299]

CD95 expression is found on myeloid cells, fibroblasts, and activated lymphocytes, as well as on various lymphoma and leukemia cells, but is not always associated with cell death.[18,123,124,128,134,135,148,298,300,301] In addition, lymphoblastoid cells transformed with HTLV-1 and -2, HIV, or EBV also highly express functional CD95 antigen.[302-304] CD95 is also expressed in tissues such as liver, heart, lung, and ovary.[298] Detailed analyses have shown that the CD95 antigen is weakly expressed on the surface of most malignant B cells isolated from CLLs, but is upregulated by stimulation with

Staphylococcus aureus Cowan I (SAC) or IL-2 and showed CD95-mediated apoptosis.[305] In contrast, HCL B cells expressed CD95 at moderate levels.[305] The induction of CD95-mediated apoptosis was correlated in some instances with bcl-2 downregulation.[305] The bcl-2 expression is correlated with inhibition of apoptosis[306] and deregulation of bcl-2 expression might be part of the pathogenesis of lymphomas (eg, follicular lymphomas) and leukemias (eg, B-cell CLL).[307] In addition, mediastinal B-cell lymphomas coexpress, depending on their differentiation stage, CD95 and CD54.[308] Interestingly, B cells from one CLL cases did not show bcl-2 downregulation after stimulation with SAC and IL-2, and CD95 MoAb induced a proliferative signal.[305] Further, one case of B-cell lymphoma stimulated with IL-14 also showed significant growth enhancement with CD95 MoAb treatment.[309] Similarly, fresh PBTs showed a costimulatory response in the presence of CD95 MoAbs or sCD95L, but chronic activated PBTs or T-cell clones underwent apoptosis.[123,128,300,302,310-312]

CD95 expression of Burkitt's lymphoma (BL) cell lines is associated with a lymphoblastoid phenotype.[304] EBV^- and EBV^+ BL cell lines with type I phenotype ($CD10^+$, $CD21^-$, $CD23^-$, $CD30^-$, $CD39^-$, $CD70^-$, $CD77^+$) corresponding to primary BL tumor cells have no detectable CD95 surface expression. Accordingly, primary BL tumor cells (3 cases) were also $CD95^-$. In contrast, EBV^+ BL cell lines with type III lymphoblastoid phenotype ($CD10^-$, $CD21^+$, $CD23^+$, $CD30^+$, $CD39^+$, $CD70^+$, $CD77^-$) as well as normal lymphoblastoid B-cell lines expressed the CD95 antigen at high density, but 6 of 7 $CD95^+$ BL cell lines were not sensitive to CD95-mediated killing. In addition, only one of eight B-cell NHLs and one of two T-cell NHLs expressed low levels of CD95, which was upregulated by IL-14.[309] The expression of the CD95 antigen has been reported for the malignant cells of 83% (10 of 12 patients) of follicular lymphoma cases and 56% (18 of 32 patients) of diffuse lymphoma cases.[313] Subgrouping into different histiologic subgroups showed similar distribution for all categories.[313] In addition, all adult T-cell leukemia cases ($n = 12$) were $CD95^+$ and underwent apoptosis by adding CD95 MoAbs.[314] The detailed functional role of CD95 for cell survival and tumor growth is presently not well understood. Recently, the cognate for CD95 (CD95L/FASL) has been cloned and characterized as a 31-kD type II transmembrane protein with 25% to 30% homology to other members of the TNF ligand superfamily.[47] Recombinant CD95L exists also in a soluble form, with similar biologic activities seen for the CD95 MoAbs or membrane-bound CD95L.[47] The physiologic presence and role of the sCD95L remain to be determined.

CD95 and CD95L expression and function has not been well investigated for HD, but the HD-derived cell lines HDLM-2, KM-H2, L-428, and L-540 express CD95 and show apoptosis after treatment with CD95 MoAbs or soluble CD95L (H.J.G., manuscript in preparation).

In general, CD95 shares a dual role with the ability to mediate stimulatory or inhibitory/cytotoxic signals depending on the target cells or activation stage. The CD95/CD95L-mediated T-cell cytotoxicity could play a major role in controlling the immune response of peripheral lymphocytes and might be involved in T-cell tolerance.[315,316] In general, the cloning of the CD95L will further improve our understanding of the mechanisms of apoptosis and the functional relevance of CD95(FAS)-CD95L(FASL) interaction for malignancies, particular lymphoid tumors, such as a variety of lymphomas and leukemias with B- and T-cell phenotype.

4-1BB/4-1BBL INTERACTION AND THE PATHOGENESIS OF LYMPHOMAS

4-1BB was identified and cloned from activated T cells (activation induced cDNA clone).[10,16,44,317,318] The 33-kD 4-1BB molecule is expressed on activated T cells ($CD4^+$ and $CD8^+$) and thymocytes.[16,317,319] 4-1BB antibodies have costimulatory activity for T-cell proliferation.[319] Initially, it was reported that extracellular matrix proteins bind 4-1BB, but the functional relevance remains unclear.[320] Subsequently, murine and human 4-1BB ligands were identified and expression cloned.[43,44] Expression of 4-1BBL was found for activated T cells, stromal cells, activated macrophages, EBV-transformed B cells, some tumor and leukemia cell lines, and a variety of tissues such as brain, placenta, lung, skeletal muscle, and kidney.[43,44,84] 4-1BBL costimulates T-cell and thymocyte proliferation, but other biologic activities for the immune system and hematopoesis that are indicated by the wide distribution pattern of 4-1BB and 4-1BBL need to be identified. It is of additional interest that signals through 4-1BB enhance activation-induced cell death (AICD) of T cells.[44] 4-1BBL is able to function as a signal transducing molecule.[84] 4-1BB and 4-1BBL are expressed on activated T cells and could play an autocrine regulatory role in T-cell interaction.[43,44,84] Primary lymphomas have not been analyzed for 4-1BB and 4-1BBL expression, but a series of HD-derived cell lines express, in addition to the TNFRs, CD30, CD40, and FAS also 4-1BB (H.J.G., manuscript in preparation). The functional and pathologic relevance needs to be examined.

THE OX40 MOLECULE AND HD

The OX40 molecule was originally described as a cell surface antigen on activated rat T cells[321]; subsequently, the genes encoding rat, mouse, and human OX40 have been cloned.[45,46,322-324] Expression of OX40 was reported initially to be restricted to activated $CD4^+$ T cells.[322,323] The human OX40 molecule is identical to the ACT35 antigen, described as strictly activation-associated antigen.[162,324] No expression was found for resting peripheral blood lymphocytes, peripheral blood B cells, and thymocytes. In lymphoid tissue, huOX40 expression was seen for scattered cells in the interfollicular zone, the follicular mantle zone, and the germinal centers.[162] Tissue macrophages were more weakly positive. For HD, only a few cases showed OX40 expression of H-RS cells, but T cells surrounding H-RS cells in a rosette fashion were strongly positive.[162] Recently, murine and human OX40L have been cloned from the murine lymphoma cell line S49.1 or the activated B-lymphoblastoid cell line MSAB, respectively, and the human homoloque identified to be gp34, a protein expressed on HTLV-1–infected human

leukemic T cells.[45,46,325,326] OX40L expression is, as the OX40 receptor, selectively induced on activated $CD4^+$ and $CD8^+$ T cells, but not on B cells.[45] The OX40L is also expressed on HTLV-1–transformed cell lines, stimulated B-lymphoblastoid cell lines, and THP-1 cells.[46] As predicted, human OX40L costimulates T-cell proliferation and cytokine production (eg, IL-2 and IL-4) as part of the regulatory cascade for immune resonses.[45,46] A possible correlation between the OX40/OX40L system and virally induced pathogenesis needs further evaluation. Detailed expression and functional analyses have to be performed to understand the role of OX40-OX40L for the pathogenesis and/or tumorigenesis of lymphomas, particularly in the context of viral transformation (eg, EBV, HTLV, and HIV).

TNF AND LT EXPRESSION IN HD WITH UNCLEAR BIOLOGIC RELEVANCE

TNF was originally defined by its antitumor activity but is also a major mediator of inflammation and cellular immune response.[8] TNF was found to be cytotoxic to a number of transformed cell lines in vitro.[327] TNF induces cachexia in LPS-treated mice with profound effects on general cellular metabolism and development of weight loss, fever, acute phase reaction, infection, or neoplasia.[328] TNF enhances the proliferation of T cells, modulates T-cell receptor expression, enhances NK cell activity, and regulates human B-cell function. TNF also has marked effects on neutrophils, eosinophil recruitment, monocyte/macrophage activation, fibroblast growth stimulation, and endothelial cell/leukocyte interactions.[329] TNF is produced by many cell types, including monocytes/macrophages, lymphocytes, and fibroblasts.[8] Activated macrophages have the highest TNF production.[328]

LT is a cytokine structurally related to TNF with approximately 50% sequence homology, the same chromosomal localization, and trimer structure.[8] LT is synthesized primarily by T cells, although some EBV-transformed B-cell lines and tonsil B cells produce it.[8] LT and TNF have similar but not identical inflammatory and immunomodulatory activities.[8] LT is often less potent than TNF. Initially, LT (TNF-β) was cloned as a soluble cytokine.[330] Surface LT does not result from the presence of the transmembrane region but, rather, was found associated with a 33-kD integral membrane glycoprotein.[75,76] The cloned gene encoding this second protein in the surface LT complex was found to be a new member of the TNF ligand superfamily.[35] Recently, the LT complex units have been renamed as LTα (TNF-β/LT) and LTβ (p33), being synthesized in soluble form as a LTα homotrimer or as a membrane-anchored heterotrimeric complex composed of LTα and LTβ units (eg, $\beta_2\alpha_1$).[35] Generic ligand-receptor interaction analysis predicts that the heterotrimeric LTβ complex would produce a functionally inactive receptor-binding protein.[35,77] The immunologic function and biologic role of LTβ is presently not well understood.

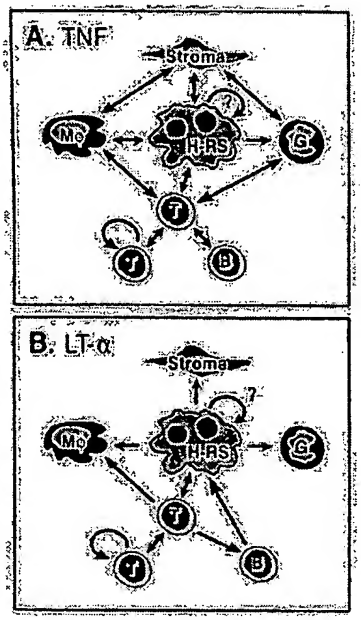
Receptors for TNF and LT are expressed at low levels on most tissues and various cell types.[8,331] Three distinct receptors have been shown to bind TNF, LTα, and LTβ.[9-11,77] The p60 TNFR type I and p80 TNFR type II cDNAs encode distinct proteins with 20% homology in the ligand-binding domain (extracellular domain); both receptors bind TNF and LTα with similar affinities.[9-11] The heterotrimer LTβ binds to the recently identified TNFR-RP surface protein (TNFR-III).[12,77] Expression of TNFRs on human PBT cells is activation dependent.[332] TNFR expression is upregulated by agents such as IL-2, IFN-α, cAMP, and different hormones but is downregulated by IL-1, PMA, glucocorticoids, and LPS.[8] Soluble forms of TNFR-I and TNFR-II have been identified in the serum of normal persons and tumor patients.[91,94,95,97] It is of particularly interest that several viral ORFs such as SFV-T2, MYX-T2, G4R, crmB, Va53, and SalF19R, encode soluble homologues of the TNFR proteins with a possible role in viral host response.[10,20-22,333,334] These viral ORFs show a novel mechanism of viral subversion of the host immune response.[67]

Expression of TNF and LTα protein and mRNA has been reported for a series of HD-derived cell lines.[196,258,335-338] Similarly, TNF expression of H-RS cells was shown in primary tissue from HD patients.[175,265,336,337,339,340] Using immunohistochemistry and/or in situ hybridization, 56 of 104 cases investigated (54% positive cases) showed strong cytoplasmic TNF signals for H-RS cells. In addition, immunoreactivity for TNF was observed for some macrophages. Furthermore, abundant LTα expression was found on primary H-RS cells for 47 of 59 HD cases analyzed (80% of cases positive).[175,339] In comparison with TNF, the LTα-specific mRNA signals were usually of higher intensity and present in larger proportions of H-RS cells.[175] TNF and LTα gene transcripts were also seen in some lymphoid cells.

Most HD-derived cell lines express the p60 and p80 TNFRs on their surface or at the mRNA level.[196,335] The presence of TNFR-RP protein on cultured or primary H-RS cells has not been investigated. Limited data are available for the TNFR expression in HD cases. One study reported the expression of p60 TNFR in 1 of 4 (25%) and p80 TNFR in 2 of 4 (50%) HD cases analyzed using immunohistochemistry.[341] Further detailed expression studies for primary HD material are required to fully understand the involvement of TNF and LTα for the pathogenesis of HD, including growth control for H-RS cells.

These different studies indicate that TNF and LTα expression of the malignant H-RS cells in HD-involved tissue is a frequent finding and could have a central functional relevance for oncogenesis and pathogenesis of HD (Fig 8). High-level expression of LTα for cultured and primary H-RS cells is a striking feature for HD, with unknown biologic correlation.[64] Primary and cultured H-RS cells express not only TNF and LTα but also the TNFRs, suggesting a possible autocrine growth loop for H-RS cells (Fig 8). TNF and LTα lack mitogenic activity on cultured H-RS cells and a presumed autocrine growth loop, based on coexpression of corresponding ligands and receptors, has so far not been shown.[342] It is of interest, that recombinant CD30L and CD40L induce the secretion of TNF and LTα.[117,258]

A number of typical pathologic and clinical features of HD are consistent with characteristics of a tumor of cytokine-producing cells, including occurence of B symptoms, sclerosis, eosinophilia, acute-phase responses, T-cell rosetting and activation, impaired immune responses, and generalized itching.[54] Furthermore, cytokines produced by H-RS cells



1. Enhancement of T cell proliferation, antigen expression, and cytokine production
2. Activation of granulocyte and eosinophil recruitment
3. Stimulation of monocytes/macrophages
4. Fibroblast (stroma) growth stimulation with enhanced collagen formation
5. Involvement in control of H-RS cell growth (paracrine and/or autocrine loops), cytokine secretion and activation
6. "Central" cytokine for typical clinical and pathological presentations of HD, including acute phase response and B symptoms

Fig 8. TNF and LTα involvement in the interaction between H-RS cells and surrounding bystander cells. TNF (A) is produced by many cell types, such as monocytes/macrophages, lymphocytes, and stroma cells. Expression of TNF and TNFRs is a common finding for H-RS cells. TNF involvement in paracrine and/or autocrine growth stimulation of H-RS cells remains unclear. TNF could also be critical for accumulation/activation of bystander cells and one mediator for systemic B symptoms. Similarly, LTα (B) is frequently overexpressed by H-RS cells in HD. In contrast to TNF, LTα expression is mainly restricted to lymphoid cells. The functional role of LTα for HD is presently unclear.

might interact with surrounding bystander cells, particularly T cells; conversely, H-RS cells might respond to cytokines produced by surrounding normal reactive bystander cells. TNF and LTα are part of these deregulated cytokine network involved in HD. TNF and LTα could be involved in causing fever, weight loss, and night sweats, as cytokines involved in the development of the constitutional B symptoms.[328] TNF is also mitogenic for fibroblasts and induces collagen synthesis with a potential role in formation of sclerosis.[329] Elevation of, eg, fibrinogen or prostaglandin serum levels, frequently seen in HD, could be associated with TNF secretion.[54,343] It is of interest that HD patients have elevated TNF serum levels (47 of 76 HD patients [62%]); the extent of increase correlated with disease stage and the presence or absence of B symptoms.[344] Similarly, the mean serum levels of the soluble p60 TNFR were significantly higher in HD patients than in healthy controls.[344] The degree of increase correlated with TNF serum levels, disease stage, and disease activity (presence of B symptoms). Furthermore, increased soluble p60 TNFR serum levels of HD patients in remission could be involved in the cellular immune defect characteristic for HD patients.[345,346] The elevated soluble TNFR concentration might support the escape of H-RS cells in the predisposing immunocompromised host from the tumor-suppressive effects of TNF and LTα and also cellular antitumor immune response by blocking appropriate T-cell activation and function. In addition, systemic TNF might be causative with other immune modulators, such as IL-1 and IL-6, in development of B symptoms and/or metabolic wasting (Fig 8).

TNF AND TNFR INTERACTION FOR NHLS

Immunohistochemical studies of nonmalignant, reactive cells showed that TNF-positive cells were rarely detected in lymph nodes with activation of the B-cell compartment but were frequently detected in sections from patients with diffuse or mixed lymphadenitis with expansion of the T-cell–dependent areas.[265,347,348] As presumed, strong TNF signals were associated with the presence of macrophages. The analysis of 20 NHL cases showed only 4 of 16 B-cell NHL cases showed weak scattered TNF-positive cells.[265] In the four T-cell NHLs, TNF was not detectable on the neoplastic lymphoma cells but was detectable on macrophages in T-cell paracortical areas.[265] In contrast to the high frequency of TNF and, particularly, the LTα expression of the neoplastic H-RS cells in HD, the neoplastic lymphoma cells of NHLs seems only rarely to produce TNF and LTα as an indicator of their malignant transformation. Similarly, RNA prepared from nonmalignant, reactive lymph nodes confirmed low expression of TNF and LTα.[339] In contrast, moderate to abundant levels of TNF mRNA were detected in 12 of 35 NHL specimens (33% of cases positive; 9 low-grade and 3 high-grade NHLs).[339] In contrast, in situ hybridization with IL-6, TNF, and LTα probes of eight lymphoplasmacytic lymphomas lacked IL-6, TNF, and LTα expression.[175] Variable amounts of increased LTα mRNA were detected in 19 of 35 NHL specimens (54% of cases positive).[339] Ten of the NHL cases coexpressed TNF and LTα mRNA.[339] Interestingly, 8 of 12 lymphoma patients (67% of cases) with the presence of systemic B symptoms had high TNF mRNA levels and 11 of these 12 lymphoma cases (92% of cases) had also abundant LTα mRNA expression.[339] High TNF and LTα mRNA expression correlated significantly with the presence of systemic B symptoms. Similarly, murine and human NHL cell lines express either constitutively TNF and LTα mRNA and protein or can be induced.[8,349] Further detailed studies are needed to confirm these initial results and to demonstrate the localization of TNF/LTα-expressing cells.

So far, only one study analyzed p60 and p80 TNFR expression systematically by using immunohistochemistry for normal tissues and NHL specimens.[341] For the normal

lymphoid tissue sections, p60 TNFR expression was restricted to dentritic reticulum cells of germinal centers, but p80 TNFR was found on a major cell population of interdigitating cells and activated lymphocytes in the interfollicular T-cell areas. The p60 and p80 TNFR expression sites are different and could allow different biologic function of TNF and LTα with either paracrine or autocrine signaling pathways. In reactive lymph nodes, the number of p80 TNFR-positive cells was increased in the T-cell areas.[341] The analysis of a limited number of B- and T-cell NHL sections (n = 30) showed that mainly lymphoma cells with a high-grade malignant phenotype (6 of 14 B-cell NHLs and 6 of 8 T-cell NHLs) expressed p80 but lacked p60 TNFR expression.[341]

TNF and LTα have important roles in normal B-cell activation, growth, and differentiation.[108,109,350] TNF is capable of inducing proliferation of the TNFR-positive neoplastic B cells from CLL patients.[351] Very recently, it was shown that lymphoblastoid B-cell lines and BL cell lines with a lymphoblastoid phenotype ($CD10^-$, $CD77^-$, $CD23^+$, $CD40^+$) use LTα as an autocrine growth factor and act mainly through the p60 TNFR.[352] Overall, the expression and functional relevance of the cytokines TNF and LTα for NHLs remain unclear. Malignant B cells from CLL patients use TNF as a growth factor, but similar data are presently not reported for NHLs. Further studies have to confirm the expression not only of TNF and LTα but also of the two TNFRs for different NHL entities as well as the potential biologic relevance for growth control, differentiation, and activation of NHL cells.

SUMMARY

The TNF receptor superfamily members are all type I membrane glycoproteins with typical homology in the extracellular domain of variable numbers of cysteine-rich repeats (overall homologies, 25% to 30%). In contrast, the TNF ligand superfamily members (with the exception of LTα) are type II membrane glycoproteins with homology to TNF in the extracellular domain (overall homologies, 20%). TNF and LTα are trimeric proteins and are composed of β-strands forming a β-jellyroll. The homology of the β-strand regions for the TNF ligand superfamily members suggest a similar β-sandwich structure and possible trimeric or multimeric complex formation for most or all members. A genetic linkage, as evidence for evolutionary relatedness, is found by chromosomal cluster of TNFR p80, CD30, 4-1BB, and OX40 for 1p36; TNFR p60, TNFR-RP, and CD27 for 12p13; TNF, LTα, and LTβ for 6 (MHC locus); CD27L and 4-1BBL for 19p13; and FASL and OX40L for 1q25.

Of the TNF ligand superfamily, TNF, LTα, and LTβ and their receptors (TNFR p60, TNFR p80, and TNFR-RP) interact in a complex fashion of cross-binding. However, the other family members presently have a one ligand/one receptor binding principle (CD27/CD27L, CD30/CD30L, CD40/CD40L, 4-1BB/4-1BBL, OX40/gp34, and FAS/FASL). In general, the members of the TNF ligand superfamily mediate interaction between different hematopoietic cells, such as T cell/B cell, T cell/monocyte, and T cell/T cell. Signals can be transduced not only through the receptors but also through at least some of the ligands. The transduced signals can be stimulatory or inhibitory depending on the target cell or the activation state. Taken together, TNF superfamily ligands show for the immune response an involvement in the induction of cytokine secretion and the upregulation of adhesion molecules, activation antigens, and costimulatory proteins, all known to amplify stimulatory and regulatory signals. On the other hand, differences in the distribution, kinetics of induction, and requirements for induction support a defined role for each of the ligands for T-cell–mediated immune responses. The shedding of members of the TNF receptor superfamily could limit the signals mediated by the corresponding ligands as a functional regulatory mechanism. Induction of cytotoxic cell death, observed for TNF, LTα, CD30L, CD95L, and 4-1BBL, is another common functional feature of this cytokine family. Further studies have to identify unique versus redundant biologic and physiologic functions for each of the TNF superfamily ligands.

Primary H-RS cells can express TNF, LTα, and CD27L but not CD30L and CD40L, in addition to IL-1α, IL-5, IL-6, IL-9, and M-CSF. In addition, H-RS cells express high copy numbers of several cytokine receptors such as IL-2R (p55, p75, and p64 subunits), IL-6R, M-CSFR (c-fms), SCFR (c-kit), CD30, CD40, and TNFRs.[64] Cytokines produced by H-RS cells might support the growth of tumor cells (autocrine growth loop) and/or interact with surrounding reactive bystander cells, particularly T cells. Conversely, H-RS cells might respond to cytokines produced by surrounding reactive normal cells (paracrine growth loop). The different interactions between H-RS cells and surrounding normal, reactive bystander cells, such as lymphocytes, plasma cells, histiocytes, neutrophils, eosinophils, and stromal cells, is characteristic for HD. The expression and biologic effects of a panel of cytokines and their counterpart receptors seem to be involved in the pathobiologic interaction between H-RS cells and particularly lymphocytes, mainly $CD4^+$ T cells. Detailed analyses have to verify the predicted biologic activities of TNF, LTα, CD27L, CD30L, CD40L, 4-1BBL, gp34/OX40L, and FASL for the H-RS cell/T-cell interactions with impact on tumor growth and pathogenesis of HD. Cytokines and cytokine receptors, including TNF/TNFRs, CD30/CD30L, and CD40/CD40L, are clearly critical elements in the pathology of HD and are part of the deregulated network of interactive signals between H-RS cells and surrounding bystander cells with membrane-associated and cytokine-mediated events. HD is a tumor of cytokine-producing cells that is causative for several characteristic clinical and pathologic presentation of HD.

The functional role of cytokines for the pathogenesis of NHLs is presently unclear. Malignant NHL cells express, depending on their immunophenotype, several TNF receptor and ligand superfamily members. B-cell NHLs are frequently CD27/CD27L, CD30 or CD30L, CD40, and TNFRs/TNF positive, but T-cell NHLs have expression of CD30, CD40L, and TNFRs/TNF. Further functional analysis will increase our understanding of the involvement of TNF superfamily ligands in the pathogenesis of NHLs.

Several TNFR superfamily members could be candidates for novel treatment protocols. Recombinant CD30L and CD40L could be by itself antitumorigenic for $CD30^+$ ALCLs

and CD40$^+$ B-cell NHLs, respectively. Furthermore, CD30 and CD40 might be used for tumor targeting after conjugation with radioisotypes or cytostatic drugs for CD30$^+$ and/or CD40$^+$ HD and NHLs.

REFERENCES

1. Williams AF, Barclay AN: The immunoglobulin superfamily-domains for cell surface recognition. Annu Rev Immunol 6:381, 1988
2. Mallett S, Barclay AN: A new superfamily of cell surface proteins related to the nerve growth factor receptor. Immunol Today 12:220, 1991
3. Cosman D: The hematopoietin receptor superfamily. Cytokine 5:95, 1993
4. Armitage RJ: Tumor necrosis factor receptor superfamily members and their ligands. Curr Opin Immunol 6:407, 1994
5. Smith CA, Farrah T, Goodwin RG: The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 75:959, 1994
6. Kishimoto T, Taga T, Akira S: Cytokine signal transduction. Cell 76:253, 1994
7. Johnson D, Lanahan A, Buck CR, Sehgal A, Morgan C, Mercer E, Bothwell M, Chao M: Expression and structure of the human NGF receptor. Cell 47:545, 1986
8. Beutler B: Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine. New York, NY, Raven, 1992
9. Loetscher H, Pan Y-CE, Lahm H-W, Gentz R, Brockhaus M, Tabuchi H, Lesslauer W: Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. Cell 61:351, 1990
10. Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzy R, Dower SK, Cosman D, Goodwin RG: A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 248:1019, 1990
11. Schall TJ, Lewis M, Koller KJ, Lee A, Rice GC, Wong GH, Gatanaga T, Granger GA, Lentz R, Raab H, Kohr KJ, Goeddel DV: Molecular cloning and expression of a receptor for human tumor necrosis factor. Cell 61:361, 1990
12. Baens M, Chaffanet M, Cassiman J-J, van den Berghe H, Marynen P: Construction and evaluation of a hncDNA library of human 12p transcribed sequences derived from a somatic cell hybrid. Genomics 16:214, 1993
13. Camerini D, Walz G, Loenen WAM, Borst J, Seed B: The T cell activation antigen CD27 is a member of the nerve growth factor/tumor necrosis factor receptor gene family. J Immunol 147:3165, 1991
14. Dürkop H, Latza U, Hummel M, Eitelbach F, Seed B, Stein H: Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell 68:421, 1992
15. Stamenkovic I, Clark EA, Seed B: A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. EMBO J 8:1403, 1989
16. Kwon BS, Weissman SM: cDNA sequences of two inducible T-cell genes. Proc Natl Acad Sci USA 86:1963, 1989
17. Mallet S, Fossum S, Barclay AN: Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes—A molecule related to nerve growth factor receptor. EMBO J 9:1063, 1990
18. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S-I, Sameshima M, Hase A, Seto Y, Nagata S: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 66:233, 1991
19. Oehm A, Behrmann I, Falk W, Pawlita M, Maier G, Klas C, Li-Weber M, Richards S, Dhein J, Trauth BC, Postingl H, Krammer, PH: Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen. J Biol Chem 267:10709, 1992
20. Smith CA, Davis T, Wignall JM, Din WS, Farrah T, Upton C, McFadden G, Goodwin RG: T2 open reading frame from the shope fibroma virus encodes a soluble form of the TNF receptor. Biochem Biophys Res Commun 176:335, 1991
21. Howard ST, Chan YS, Smith GL: Vaccinia virus homologues of the shope fibroma virus inverted terminal repeat proteins and a discontinuous ORF related to the tumor necrosis factor receptor family. Virology 180:633, 1991
22. Upton C, Macen JL, Schreiber M, McFadden G: Myxoma virus expresses a secreted protein with homology to the tumor necrosis factor receptor gene family that contributes to viral virulence. Virology 184:370, 1991
23. Welcher AA, Bitler CM, Radeke MJ, Shooter EM: Nerve growth factor binding domain of the nerve growth factor receptor. Proc Natl Acad Sci USA 88:159, 1991
24. Baldwin AN, Bitler CM, Welcher AA, Shooter EM: Studies on the structure and binding properties of the cysteine-rich domain of rat low affinity nerve growth factor receptor (p75NGFR). J Biol Chem 267:8352, 1992
25. Marsters SA, Frutkin AD, Simpson NJ, Fendly BM, Ashkenazi A: Identification of cysteine-rich domains of the type 1 tumor necrosis factor receptor involved in ligand binding. J Biol Chem 267:5747, 1992
26. Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi J-P, van Kooten C, Liu YJ, Rousset F, Saeland S: The CD40 antigen and its ligand. Annu Rev Immunol 12:881, 1994
27. Bradshaw RA, Blundell TL, Lapatto R, McDonald NQ, Murray-Rust J: Nerve growth factor revisited. Trends Biochem Sci 18:48, 1993
28. Eide FF, Lowenstein DH, Reichardt LF: Neurotrophins and their receptors—Current concepts and implications for neurologic disease. Exp Neurol 121:200, 1993
29. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV: Human tumour necrosis factor: Precursor structure, expression and homology to lymphotoxin. Nature 312:724, 1984
30. Old LJ: Tumor necrosis factor (TNF). Science 230:630, 1985
31. Wang AM, Creasey AA, Ladner MB, Lin LS, Strickler J, Van Arsdell JN, Yamamoto R, Mark DF: Molecular cloning of the complementary DNA for human tumor necrosis factor. Science 228:149, 1985
32. Beutler B, Cerami A: Cachectin and tumour necrosis factor as two sides of the same biological coin. Nature 320:584, 1986
33. Gray PW, Aggarwal BB, Benton CV, Bringman TS, Henzel WJ, Jarrett JA, Leung DW, Moffat B, Ng P, Svedersky LP, Palladino MA, Nedwin GE: Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity. Nature 312:721, 1984
34. Aggarwal BB, Henzel WJ, Moffat B, Kohr WJ, Harkins RN: Primary structure of human lymphotoxin derived from 1788 lymphoblastoid cell line. J Biol Chem 260:2334, 1985
35. Browning JL, Ngam-ek A, Lawton P, DeMarinis J, Tizard R, Chow EP, Hession C, O'Brien-Greco B, Foley SF, Ware CF: Lymphotoxin β, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. Cell 72:847, 1993
36. Goodwin RG, Alderson MR, Smith CA, Armitage RJ, VandenBos T, Jerzy R, Tough TW, Schoenborn MA, Davis-Smith T, Hennen K, Falk B, Cosman D, Baker E, Sutherland GR, Grabstein KH, Farrah T, Giri JG, Beckmann MP: Molecular and biological

characterization of a ligand for CD27 defines a new family of cytokines with homology to tumor necrosis factor. Cell 73:447, 1993

37. Smith CA, Gruss H-J, Davis T, Anderson D, Farrah T, Baker E, Sutherland GR, Brannan CI, Copeland NG, Jenkins NA, Grabstein KH, Gliniak B, McAlister IB, Fanslow W, Alderson M, Falk B, Gimpel S, Gillis S, Din WS, Goodwin RG, Armitage RJ: CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 73:1349, 1993

38. Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, Anderson DM, Gimpel SD, Davis-Smith T, Maliszewski CR, Clark EA, Smith CA, Grabstein KH, Cosman D, Spriggs MK: Molecular and biological characterization of a murine ligand for CD40. Nature 357:80, 1992

39. Graf D, Korthäuer U, Mages HW, Senger G, Kroczek RA: Cloning of TRAP, a ligand for CD40 on human T cells. Eur J Immunol 22:3191, 1992

40. Spriggs MK, Armitage RJ, Strockbine L, Clifford KN, Macduff BM, Sato TA, Maliszewski CR, Fanslow WC: Recombinant human CD40 ligand stimulates B cell proliferation and immunoglobulin E secretion. J Exp Med 176:1543, 1992

41. Hollenbaugh D, Grosmaire LS, Kullas CD, Chalupny NJ, Braesch-Andersen S, Noelle RJ, Stamenkovic I, Ledbetter JA, Aruffo A: The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: Expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J 11:4313, 1992

42. Gauchat JF, Aubry JP, Mazzei G, Life P, Jomotte T, Elson G, Bonnefoy JY: Human CD40-ligand: Molecular cloning, cellular distribution and regulation of expression by factors controlling IgE production. FEBS Lett 315:259, 1993

43. Goodwin RG, Din WS, Davis-Smith T, Anderson DM, Gimpel SD, Sato TA, Maliszewski CR, Brannan CI, Copeland NG, Jenkins NA, Farrah T, Armitage RJ, Fanslow WC, Smith CA: Molecular cloning of a ligand for the inducible T-cell gene 4-1BB: A member of an emerging family of cytokines with homology to tumor necrosis factor. Eur J Immunol 23:2631, 1993

44. Alderson MR, Smith CA, Tough TW, Davis-Smith T, Armitage RJ, Falk B, Roux E, Baker E, Sutherland GR, Din WS, Goodwin RG: Molecular and biological characterization of human 4-1BB and its ligand. Eur J Immunol 24:2219, 1994

45. Baum PR, Gayle RB III, Ramsdell F, Srinivasan S, Sorensen RA, Watson ML, Seldin MF, Baker E, Sutherland GR, Clifford KN, Alderson MR, Goodwin RG, Fanslow WC: Molecular characterization of murine and human OX40/OX40 ligand systems: Identification of a human OX40 ligand as the HTLV-1 regulated protein gp34. EMBO J 13:3992, 1994

46. Godfrey WR, Fagnoni FF, Harara MA, Buck D, Engleman EG: Identification of a human OX-40 ligand, a costimulator of $CD4^+$ T cells with homology to tumor necrosis factor. J Exp Med 180:757, 1994

47. Suda T, Takahashi T, Golstein P, Nagata S: Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 75:1169, 1993

48. Alderson MR, Tough TW, Davis-Smith T, Braddy S, Falk B, Schooley KA, Goodwin RG, Smith CA, Ramsdell F, Lynch DH: Fas ligand mediates activation-induced cell death in human T lymphocytes. J Exp Med 181:71, 1995

49. Lynch DH, Watson ML, Alderson MR, Baum PR, Miller RE, Tough T, Gibson M, Davis-Smith T, Smith CA, Hunter K, Bhat D, Din W, Goodwin RG, Seldin MF: The mouse Fas-ligand gene is mutated in *gld* mice and is part of a TNF family gene cluster. Immunity 1:131, 1994

50. Takahashi T, Tanaka M, Brannan CI, Jenkins NA, Copeland NG, Suda T, Nagata S: Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. Cell 76:969, 1994

51. Kriegler M, Perez C, DeFay K, Albert I, Lu SD: A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: Ramifications for the complex physiology of TNF. Cell 53:45, 1988

52. Mohler KM, Sleath PR, Fitzner JN, Cerretti DP, Alderson M, Derwar SS, Torrance DS, Otten-Evans C, Greenstreet T, Weerawarna K, Kronheim SR, Petersen M, Gerhart M, Kozlosky CJ, March CJ, Black RA: Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. Nature 370:218, 1994

53. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC, Grogan TM, Isaacson PG, Knowles DM, Mason DY, Müller-Hermelink H-K, Pileri SA, Piris MA, Ralfkiaer E, Warnke RA: A revised European-American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. Blood 84:1361, 1994

54. Kaplan HS: Hodgkin's Disease. Boston, MA, Harvard, 1980

55. Kaufman D, Longo DL: Hodgkin's disease. Crit Rev Oncol Hematol 13:135, 1992

56. Haluska FG, Brufsky AM, Canellos GP: The cellular biology of the Reed-Sternberg cell. Blood 84:1005, 1994

57. Lukes R, Butler J, Hicks E: Natural history of Hodgkin's disease as related to its pathological picture. Cancer 19:317, 1966

58. Hansen NE, Karle H: The elusive Hodgkin cell. Scand J Haematol 26:353, 1981

59. Jones DD: The histogenesis of the Reed-Sternberg cell and its mononuclear counterparts. J Pathol 151:191, 1987

60. Jaffe ES: The elusive Reed-Sternberg cell. N Engl J Med 320:529, 1989

61. Drexler HG: Recent results on the biology of Hodgkin and Reed-Sternberg cells. I. Biopsy material. Leuk Lymphoma 8:283, 1992

62. Drexler HG: Recent results on the biology of Hodgkin and Reed-Sternberg cells. II. Continuous cell lines. Leuk Lymphoma 9:1, 1993

63. Herbst H, Stein H, Niedobitek G: Epstein-Barr virus and CD30+ malignant lymphomas. Crit Rev Oncog 4:191, 1993

64. Gruss H-J, Herrmann F, Drexler HG: Hodgkin's disease: A cytokine producing tumor. Crit Rev Oncog 5:23, 1995

65. Spriggs MK, Fanslow WC, Armitage RJ, Belmont J: The biology of the human ligand for CD40. J Clin Immunol 13:373, 1993

66. Farrah T, Smith CA: Emerging cytokine family. Nature 358:26, 1992

67. Gooding LR: Virus proteins that counteract host immune defenses. Cell 71:5, 1992

68. Howard OMZ, Clouse KA, Smith C, Goodwin RG, Farrar WL: Soluble tumor necrosis factor receptor: Inhibition of human immunodeficiency virus activation. Proc Natl Acad Sci USA 90:2335, 1993

69. Hu F-Q, Smith CA, Pickup DJ: Cowpox virus contains two copies of an early gene encoding a soluble secreted form of the type II TNF receptor. Virology 204:343, 1994

70. Smith GL: Vaccinia virus glycoproteins and immune evasion. J Gen Virol 74:1725, 1993

71. Jones EY, Stuart DI, Walker NP: The three-dimensional structure of tumor necrosis factor. Prog Clin Biol Res 349:321, 1990

72. Eck MJ, Ultsch M, Rinderknecht E, de Vos AM, Sprang SR: The structure of human lymphotoxin (tumor necrosis factor-β) at 1.9-Å resolution. J Biol Chem 267:2119, 1992

73. Banner DW, D'Arcy A, Janes W, Gentz R, Schoenfeld H-J, Broger C, Loetscher H, Lesslauer W: Crystal structure of the soluble

human 55 kd TNF receptor-human TNFβ complex: Implications for TNF receptor activation. Cell 73:431, 1993

74. D'Arcy A, Banner DW, Janes W, Winkler FK, Loetscher H, Schonfeld HJ, Zulauf M, Gentz R, Lesslauer W: Crystallization and preliminary crystallographic analysis of a TNF-beta-55 kDa TNF receptor complex. J Mol Biol 229:555, 1993

75. Browning JL, Androlewicz MJ, Ware CF: Lymphotoxin and an associated 33-kDa glycoprotein are expressed on the surface of an activated human T cell hybridoma. J Immunol 147:1230, 1991

76. Androlewicz MJ, Browning JL, Ware CF: Lymphotoxin is expressed as a heteromeric complex with a distinct 33-kDa glycoprotein on the surface of an activated human T cell hybridoma. J Biol Chem 267:2542, 1992

77. Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, Browning JL, Din WS, Goodwin RG, Smith CA: A lymphotoxin-β-specific receptor. Science 264:707, 1994

78. Tartaglia LA, Ayres TM, Wong GHW, Goeddel DV: A novel domain within the 55 kd TNF receptor signals cell death. Cell 74:845, 1993

79. Tartaglia LA, Rothe M, Hu Y-F, Goeddel DV: Tumor necrosis factor's cytotoxic activity is signaled by the p55 TNF receptor. Cell 73:213, 1993

80. Wong GHW, Goeddel DV: Fas antigen and p55 TNF receptor signal apoptosis through distinct pathways. J Immunol 152:1751, 1994

81. Heller RA, Kronke M: Tumor necrosis factor receptor-mediated signaling pathways. J Cell Biol 126:5, 1994

82. Cayabyab M, Phillips JH, Lanier LL: CD40 preferentially costimulates activation of $CD4^+$ lymphocytes. J Immunol 152:1523, 1994

83. Bowman MR, Crimmins MAV, Yetz-Aldape J, Kriz R, Kelleher K, Herrmann S: The cloning of CD70 and its identification as the ligand for CD27. J Immunol 152:1756, 1994

84. Pollok KE, Kim YJ, Hurtado J, Zhou Z, Kim KK, Kwon BS: 4-1BB T-cell antigen binds to mature B cells and macrophages, and costimulates anti-μ-primed splenic B cells. Eur J Immunol 24:367, 1994

85. Beutler B, Cerami A: Tumor necrosis, cachexia, shock, and inflammation: A common mediator. Annu Rev Biochem 57:505, 1988

86. Beutler B, Cerami A: The biology of cachectin/TNF—A primary mediator of the host response. Annu Rev Immunol 7:625, 1989

87. Smith RA, Baglioni C: The active form of tumor necrosis factor is a trimer. J Biol Chem 262:6951, 1987

88. Eck MJ, Sprang SR: The structure of tumor necrosis factor-α at 2.6 Å resolution. Implications for receptor binding. J Biol Chem 264:17595, 1989

89. Peitsch MC, Jongeneel CV: A 3-D model for the CD40 ligand predicts that it is a compact trimer similar to the tumor necrosis factors. Int Immunol 5:233, 1993

90. Hintzen RQ, Lens SMA, Koopman G, Pals ST, Spits H, Van Lier RAW: CD70 represents the human ligand for CD27. Int Immunol 6:477, 1994

91. Engelmann H, Aderka D, Rubinstein M, Rotman D, Wallach D: A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. J Biol Chem 264:11974, 1989

92. Josimovic-Alasevic O, Dürkop H, Schwarting R, Backe E, Stein H, Diamantstein T: Ki-1 (CD30) antigen is released by Ki-1-positive tumor cells in vitro and in vivo. I. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. Eur J Immunol 19:157, 1989

93. Engelmann H, Novick D, Wallach D: Two tumor necrosis factor binding proteins purified from human urine. Evidence for immunological cross reactivity with cell surface tumor necrosis factor receptors. J Biol Chem 265:1531, 1990

94. Kohno T, Brewer MT, Baker SL, Schwartz PE, King MW, Hale KK, Squires CH, Thompson RC, Vannice JL: A second tumor necrosis factor receptor gene product can shed a naturally occurring tumor necrosis factor inhibitor. Proc Natl Acad Sci USA 87:8331, 1990

95. Lantz M, Gullberg U, Nilsson E, Olsson I: Characterization in vitro of a human tumor necrosis factor-binding protein. A soluble form of a tumor necrosis factor receptor. J Clin Invest 86:1396, 1990

96. Porteu F, Nathan C: Shedding of tumor necrosis factor receptors by activated human neutrophils. J Exp Med 172:599, 1990

97. Aderka D, Englemann H, Hornik V, Skornick Y, Levo Y, Wallach D, Kushtai G: Increased serum levels of soluble receptors for tumor necrosis factor in cancer patients. Cancer Res 51:5602, 1991

98. de Jong R, Loenen WAM, Brouwer M, van Emmerik L, de Vries EFR, Borst J, Van Lier RAW: Regulation of expression of CD27, a T cell-specific member of a novel family of membrane receptors. J Immunol 146:2488, 1991

99. Hintzen RQ, de Jong R, Hack CE, Chamuleau M, de Vries EFR, ten Berge IJM, Borst J, van Lier RAN: A soluble form of the human T cell differentiation antigen CD27 is released after triggering of the TCR/CD3 complex. J Immunol 147:29, 1991

100. Hintzen RQ, van Lier RA, Kuijpers KC, Baars PA, Schaasberg W, Lucas CJ, Polman CH: Elevated levels of a soluble form of the T cell activation antigen CD27 in cerebrospinal fluid of multiple sclerosis patients. J Neuroimmunol 35:211, 1991

101. Loenen WA, De Vries E, Gravestein LA, Hintzen RQ, Van Lier RA, Borst J: The CD27 membrane receptor, a lymphocyte-specific member of the nerve growth factor receptor family, gives rise to a soluble form by protein processing that does not involve receptor endocytosis. Eur J Immunol 22:447, 1992

102. van Kooten C, Gaillard C, Galizzi J-P, Hermann P, Fossiez F, Banchereau J, Blanchard D: B cells regulate expression of CD40 ligand on activated T cells by lowering the mRNA level and through the release of soluble CD40. Eur J Immunol 24:787, 1994

103. Cheng J, Zhou T, Liu C, Shapiro JP, Brauer MJ, Kiefer MC, Barr PJ, Mountz JD: Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science 263:1759, 1994

104. Armitage RJ, Tough TW, Macduff BM, Fanslow WC, Spriggs MK, Ramsdell F, Alderson MR: CD40 ligand is a T cell growth factor. Eur J Immunol 23:2326, 1993

105. Gruss HJ, Hirschstein D, Alderson MA, Armitage RJ: Regulation of CD30 ligand expression on peripheral blood T cells: Functional involvement in T-T cell interaction. J Immunol (in press)

106. Parker DC: T cell-dependent B cell activation. Annu Rev Immunol 11:331, 1993

107. Clark EA, Ledbetter JA: How B and T cells talk to each other. Nature 367:425, 1994

108. Kehrl JH, Alvarez-Mon M, Delsing GA, Fauci AS: Lymphotoxin is an important T cell-derived growth factor for human B cells. Science 238:1144, 1987

109. Aversa G, Punnonen J, de Vries JE: The 26-kD transmembrane form of tumor necrosis factor α on activated CD4+ T cell clones provides a costimulatory signal for human B cell activation. J Exp Med 177:1575, 1993

110. Boussiotis VA, Nadler LM, Strominger JL, Goldfeld AE: Tumor necrosis factor alpha is an autocrine growth factor for normal human B cells. Proc Natl Acad Sci USA 91:7007, 1994

111. Marlin SD, Springer TA: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 51:813, 1987

112. Linsley PS, Clark EA, Ledbetter JA: T-cell antigen CD28

mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc Natl Acad Sci USA 87:5031, 1990

113. Barrett TB, Shu G, Clark EA: CD40 signaling activates CD11a/CD18 (LFA-1)-mediated adhesion in B cells. J Immunol 146:1722, 1991

114. Rothlein R, Czajkowski M, O'Neill MM, Marlin SD, Mainolfi E, Merluzzi VJ: Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by proinflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol 141:1665, 1988

115. Björck P, Elenström-Magnusson C, Roséen A, Severinson E, Paulie S: CD23 and CD21 function as adhesion molecules in homotypic aggregation of human B lymphocytes. Eur J Immunol 23:1771, 1993

116. Clark EA, Lane PJL: Regulation of human B-cell activation and adhesion. Annu Rev Immunol 9:97, 1991

117. Gruss HJ, Braddy S, Ulrich D, Armitage RJ, Dower SK: Recombinant CD30 ligand and CD40 ligand share common biological activities on Hodgkin and Reed-Sternberg cells. Eur J Immunol (in press)

118. Ranheim EA, Kipps TJ: Activated T cells induce expression of B7/BB1 on normal or leukemic B cells through a CD40-dependent signal. J Exp Med 177:925, 1993

119. Kennedy MK, Mohler KM, Shanebeck KD, Baum PR, Picha KS, Otten-Evans CA, Janeway CA Jr, Grabstein KH: Induction of B cell costimulatory function by recombinant murine CD40 ligand. Eur J Immunol 24:116, 1994

120. Yellin MJ, Sinning J, Covey LR, Sherman W, Lee JJ, Glickman-Nir E, Sippel KC, Rogers J, Cleary AM, Parker M, Chess L, Lederman S: T lymphocyte T cell-B cell-activating molecule/CD40-L molecules induce normal B cells or chronic lymphocytic leukemia B cells to express CD80 (B7/BB-1) and enhance their costimulatory activity. J Immunol 153:666, 1994

121. Liu CC, Detmers PA, Jiang SB, Young JD: Identification and characterization of a membrane-bound cytotoxin of murine cytolytic lymphocytes that is related to tumor necrosis factor/cachectin. Proc Natl Acad Sci USA 86:3286, 1989

122. Gruss H-J, Boiani N, Williams DE, Armitage RJ, Smith CA, Goodwin RG: Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood 83:2045, 1994

123. Miyawaki T, Uehara T, Nibu R, Tsuji T, Yachie A, Yonehara S, Taniguchi N: Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. J Immunol 149:3753, 1992

124. Iwai K, Miyawaki T, Takizawa T, Konno A, Ohta K, Yachie A, Saki H, Taniguchi N: Differential expression of bcl-2 and susceptibility to anti-Fas–mediated cell death in peripheral blood lymphocytes, monocytes, and neutrophils. Blood 84:1201, 1994

125. Owen-Schaub LB, Radinsky R, Kruzel E, Berry K, Yonehara S: Anti-Fas on nonhematopietic tumors: Levels of Fas/APO-1 and bcl-2 are not predictive of biological responsiveness. Cancer Res 54:1580, 1994

126. Gillette-Ferguson I, Sidman CL: A specific intercellular pathway of apoptotic cell death is defective in the mature peripheral T cells of autoimmune lpr and gld mice. Eur J Immunol 24:1181, 1994

127. Kabelitz D, Pohl T, Pechhold K: Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. Immunol Today 14:338, 1993

128. Alderson MR, Armitage RJ, Maraskovsky E, Tough TW, Roux E, Schooley K, Ramsdell F, Lynch DH: Fas transduces activation signals in normal human T lymphocytes. J Exp Med 178:2231, 1993

129. Russell JH, Wang R: Autoimmune gld mutation uncouples suicide and cytokine/proliferation pathways in activated mature T cells. Eur J Immunol 23:2379, 1993

130. Russell JH, Rush B, Weaver C, Wang R: Mature T cells of autoimmune /pr/lpr mice have a defect in antigen-stimulated suicide. Proc Natl Acad Sci USA 90:4409, 1993

131. Bossu P, Singer GG, Andres P, Ettinger R, Marshak-Rothstein A, Abbas AK: Mature $CD4^+$ T lymphocytes from MRL/lpr mice are resistant to receptor-mediated tolerance and apoptosis. J Immunol 151:7233, 1993

132. Krammer PH, Behrmann I, Daniel P, Dhein J, Debatin KM: Regulation of apoptosis in the immune system. Curr Opin Immunol 6:279, 1994

133. Singer GG, Abbas AK: The Fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. Immunity 1:365, 1994

134. Trauth BC, Klas C, Peters AMJ, Matzku S, Möller P, Falk W, Debatin K-M, Krammer PH: Monoclonal antibody-mediated tumor regression by induction of apoptosis. Science 245:301, 1989

135. Yonehara S, Ishii A, Yonehara M: A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. J Exp Med 169:1747, 1989

136. Green DR, Scott DW: Activation-induced apoptosis in lymphocytes. Curr Opin Immunol 6:476, 1994

137. Itoh N, Nagata S: A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. J Biol Chem 268:10932, 1993

138. Grell M, Scheurich P, Meager A, Pfizenmaier K: TR60 and TR80 tumor necrosis factor (TNF)-receptors can independently mediate cytolysis. Lymphokine Cytokine Res 12:143, 1993

139. Grell M, Zimmermann G, Hulser D, Pfizenmaier K, Scheurich P: TNF receptors TR60 and TR80 can mediate apoptosis via induction of distinct signal pathways. J Immunol 153:1963, 1994

140. Heller RA, Song K, Fan N, Chang DJ: The p70 tumor necrosis factor receptor mediates cytotoxicity. Cell 70:47, 1992

141. Thoma B, Grell M, Pfizenmaier K, Scheurich P: Identification of a 60-kD tumor necrosis factor (TNF) receptor as the major signal transducing component in TNF responses. J Exp Med 172:1019, 1990

142. Tartaglia LA, Weber RF, Figari IS, Reynolds C, Palladino M Jr, Goeddel DV: The two different receptors for tumor necrosis factor mediate distinct cellular responses. Proc Natl Acad Sci USA 88:9292, 1991

143. Engelmann H, Holtmann H, Brakebusch C, Avni YS, Sarov I, Nophar Y, Hadas E, Leitner O, Wallach D: Antibodies to a soluble form of a tumor necrosis factor (TNF) receptor have TNF-like activity. J Biol Chem 265:14497, 1990

144. Tartaglia LA, Goeddel DV, Reynolds C, Figari IS, Weber RF, Fendly BM, Palladino MA Jr: Stimulation of human T-cell proliferation by specific activation of the 75-kDa tumor necrosis factor receptor. J Immunol 151:4637, 1993

145. Lewis M, Tartaglia LA, Lee A, Bennett GL, Rice GC, Wong GH, Chen EY, Goeddel DV: Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. Proc Natl Acad Sci USA 88:2830, 1991

146. Shalaby MR, Sundan A, Loetscher H, Brockhaus M, Lesslauer W, Espevik T: Binding and regulation of cellular functions by monoclonal antibodies against human tumor necrosis factor receptors. J Exp Med 172:1517, 1990

147. Rothe M, Wong SC, Henzel WJ, Goeddel DV: A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. Cell 78:681, 1994

148. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S: Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 356:314, 1992

149. Kawabe T, Naka T, Yoshida K, Tanaka T, Fujiwara H, Suematsu S, Yoshida N, Kishimoto T, Kikutani H: The immune responses in CD40-deficient mice: Impaired immunoglobulin class switching and germinal center formation. Immunity 1:167, 1994

150. Renshaw B, Fanslow WC III, Armitage RJ, Campbell KA, Liggitt D, Wright B, Davison B, Maliszewski CR: Humoral immune responses in CD40 ligand deficient mice. J Exp Med 180:1889, 1994

151. Xu J, Foy TM, Laman JD, Elliott EA, Dunn JJ, Waldschmidt TJ, Elsemore J, Noelle RJ, Flavell RA: Mice deficient for the CD40 ligand. Immunity 1:423, 1994

152. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, Bedell MA, Edelhoff S, Disteche CM, Simoneaux DK, Fanslow WC, Belmont J, Spriggs MK: CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science 259:990, 1993

153. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, Bajorath J, Grosmaire LS, Stenkamp R, Neubauer M, Roberts RL, Noelle RJ, Ledbetter JA, Francke U, Ochs HD: The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell 72:291, 1993

154. Korthäuer U, Graf D, Mages HW, Brière F, Padayachee M, Malcolm S, Ugazio AG, Notarangelo LD, Levinsky RJ, Kroczek RA: Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper IgM. Nature 361:539, 1993

155. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G: CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. Nature 361:541, 1993

156. Pfeffer K, Matsuyama T, Kündig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Kronke M, Mak TW: Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. Cell 73:457, 1993

157. Rothe J, Lesslauer W, Lötscher H, Lang Y, Koebel P, Kontgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H: Mice lacking the tumor necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature 364:798, 1993

158. Peppel K, Poltorak A, Melhado I, Jirik F, Beutler B: Expression of a TNF inhibitor in transgenic mice. J Immunol 151:5699, 1993

159. Kolls J, Peppel K, Silva M, Beutler B: Prolonged and effective blockade of tumor necrosis factor activity through adenovirus-mediated gene transfer. Proc Natl Acad Sci USA 91:215, 1994

160. De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, Smith SC, Carlson R, Shornick LP, Strauss-Schoenberger J, Russell JH, Karr R, Chaplin DD: Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science 264:703, 1994

161. McMichael AJ: Leukocyte Typing III: White Cell Differentiation Antigens. Oxford, UK, Oxford, 1986

162. Knapp W, Dörken B, Rieber EP, Stein H, Gilks WR, Schmidt RE, Kr. von dem Borne AEG: Leukocyte Typing IV: White Cell Differentiation Antigens. Oxford, UK, Oxford, 1989

163. Maurer D, Holter W, Majdic O, Fischer GF, Knapp W: CD27 expression by a distinct subpopulation of human B lymphocytes. Eur J Immunol 20:2679, 1990

164. Maurer D, Fischer GF, Fae I, Majdic O, Stuhlmeier K, von Jeney N, Holter W, Knapp W: IgM and IgG but not cytokine secretion is restricted to the CD27$^+$ B lymphocyte subset. J Immunol 148:3700, 1992

165. Sugita K, Robertson MJ, Torimoto Y, Ritz J, Schlossman SF, Morimoto C: Participation of the CD27 antigen in the regulation of IL-2–activated human natural killer cells. J Immunol 149:1199, 1992

166. van Lier RAW, Borst J, Vroom TM, Klein H, Van Mourik P, Zeijlemaker WP, Melief CJM: Tissue distribution and biochemical and functional properties of Tp55 (CD27), a novel T cell differentiation antigen. J Immunol 139:1589, 1987

167. van Lier RAW, Pool MO, Kabel P, Mous S, Terpstra F, De Rie MA, Melief CJ, Miedema F: Anti-CD27 monoclonal antibodies identify two functionally distinct subpopulations within the CD4$^+$ T cell subset. Eur J Immunol 18:811, 1988

168. Bigler RD, Bushkin Y, Chiorazzi N: S152 (CD27). A modulating disulfide-linked T cell activation antigen. J Immunol 141:21, 1988

169. Sugita K, Torimoto Y, Nojima Y, Daley JF, Schlossman SF, Morimoto C: The 1A4 molecule (CD27) is involved in T cell activation. J Immunol 147:1477, 1991

170. Martorell J, Rojo I, Vilella R, Martinez-Caceres E, Vives J: CD27 induction on thymocytes. J Immunol 145:1356, 1990

171. Hintzen RQ, de Jong R, Lens SMA, Brouwer M, Baars P, van Lier RAW: Regulation of CD27 expression on subsets of mature T-lymphocytes. J Immunol 151:2426, 1993

172. Agematsu K, Kobata T, Sugita K, Freeman GJ, Beckmann MP, Schlossman SF, Morimoto C: Role of CD27 in T cell immune response. Analysis by recombinant soluble CD27. J Immunol 153:1421, 1994

173. Hintzen RQ, Lens SM, Beckmann MP, Goodwin RG, Lynch D, van Lier RAW: Characterization of the human CD27 ligand, a novel member of the TNF gene family. J Immunol 152:1762, 1994

174. Hintzen RQ, de Jong R, Lens SMA, van Lier RAW: CD27: Marker and mediator of T-cell activation? Immunol Today 15:307, 1994

175. Foss HD, Herbst H, Oelmann E, Samol J, Grebe M, Blankenstein T, Matthes J, Qin ZH, Falini B, Pileri S, Diamantstein T, Stein H: Lymphotoxin, tumor necrosis factor and interleukin-6 gene transcripts are present in Hodgkin and Reed-Sternberg cells of most Hodgkin's disease cases. Br J Haematol 84:627, 1993

176. van Oers MH, Pals ST, Evers LM, van der Schoot CE, Koopman G, Bonfrer JM, Hintzen RQ, von dem Borne AE, van Lier RA: Expression and release of CD27 in human B-cell malignancies. Blood 82:3430, 1993

177. Schwab U, Stein H, Gerdes J, Lemke H, Kirchner H, Schaadt M, Diehl V: Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 299:65, 1982

178. Stein H, Mason DY, Gerdes J, O'Connor N, Wainscoat J, Pallesen G, Gatter K, Falini B, Delsol G, Lemke H, Schwarting R, Lennert K: The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: Evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 66:848, 1985

179. Drexler HG, Jones DB, Diehl V, Minowada J: Is the Hodgkin cell a T- or B-lymphocyte? Recent evidence from geno- and immunophenotypic analysis and in-vitro cell lines. Hematol Oncol 7:95, 1989

180. Hecht TT, Longo DL, Cossman J, Bolen JB, Hsu SM, Israel M, Fisher RI: Production and characterization of a monoclonal antibody that binds Reed-Sternberg cells. J Immunol 134:4231, 1985

181. Schwarting R, Gerdes J, Dürkop H, Falini B, Pileri S, Stein H: BER-H2: A new anti–Ki-1 (CD30) monoclonal antibody directed at a formol-resistant epitope. Blood 74:1678, 1989

182. Pfreundschuh M, Mommertz E, Meissner M, Feller AC, Hassa R, Krueger GR, Diehl V: Hodgkin and Reed-Sternberg cell associated monoclonal antibodies HRS-1 and HRS-2 react with activated cells of lymphoid and monocytoid origin. Anticancer Res 8:217, 1988

183. Bowen MA, Olsen KJ, Cheng L, Avila D, Podack ER: Functional effects of CD30 on a large granular lymphoma cell line, YT.

Inhibition of cytotoxicity, regulation of CD28 and IL-2R, and induction of homotypic aggregation. J Immunol 151:5896, 1993

184. Froese P, Lemke H, Gerdes J, Havsteen B, Schwarting R, Hansen H, Stein H: Biochemical characterization and biosynthesis of the Ki-1 antigen in Hodgkin-derived and virus-transformed human B and T lymphoid cell lines. J Immunol 139:2081, 1987

185. Nawrocki JF, Kirsten ES, Fisher RI: Biochemical and structural properties of a Hodgkin's disease-related membrane protein. J Immunol 141:672, 1988

186. Falini B, Pileri S, Pizzolo G, Dürkop H, Flenghi L, Stirpe F, Martelli MF, Stein H: CD30 (Ki-1) molecule: A new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. Blood 85:1, 1995

187. Niedobitek G, Hamilton-Dutoit S, Herbst H, Finn T, Vetner M, Pallesen G, Stein H: Identification of Epstein-Barr virus-infected cells in tonsils of acute infectious mononucleosis by in situ hybridization. Hum Pathol 20:796, 1989

188. Abbondanzo SL, Sato N, Straus SE, Jaffe ES: Acute infectious mononucleosis. CD30 (Ki-1) antigen expression and histologic correlations. Am J Clin Pathol 93:698, 1990

189. Andreesen R, Osterholz J, Lohr GW, Bross KJ: A Hodgkin cell-specific antigen is expressed on a subset of auto- and alloactivated T (helper) lymphoblasts. Blood 63:1299, 1984

190. Ellis TM, Simms PE, Slivnick DJ, Jäck H-M, Fisher RI: CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO$^+$ T cells. J Immunol 151:2380, 1993

191. Del Prete GF, De Carli M, Almerigogna F, Daniel CK, D'Elios MM, Zancuoghi G, Pizzolo G, Romagnani S: Preferrential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. FASEB J 9:81, 1995

192. Manetti R, Annunziato F, Biagiotti R, Giudizi MG, Piccinni M-P, Giannarini L, Sampognaro S, Parronchi P, Vinante F, Pizzolo G, Maggi E, Romagnani S: CD30 expression by CD8+ T cells producing type 2 helper cytokines. Evidence for large numbers of CD8+CD30+ T cell clones in human immunodeficiency virus infection. J Exp Med 180:2407, 1994

193. Del Prete G, Maggi E, Pizzolo G, Romagnani S: CD30, Th2 cytokines and HIV infection: A complex and fascinating link. Immunol Today 16:76, 1995

194. Cambiaggi A, Cantoni C, Marciano S, De Totero D, Pileri S, Tazzari PL, Stein H, Ferrini S: Cultured human NK cells express the Ki-1/CD30 antigen. Br J Haematol 85:270, 1993

195. Andreesen R, Brugger W, Löhr GW, Bross KJ: Human macrophages can express the Hodgkin's cell-associated antigen Ki-1 (CD30). Am J Pathol 134:187, 1989

196. Bargou RC, Mapara MY, Zugck C, Daniel PT, Pawlita M, Döhner H, Dörken B: Characterization of a novel Hodgkin cell line, HD-MyZ, with myelomonocytic features mimicking Hodgkin's disease in severe combined immunodeficient mice. J Exp Med 177:1257, 1993

197. Miettinen M: CD30 distribution. Immunohistochemical study on formaldehyde-fixed, paraffin-embedded Hodgkin's and non-Hodgkin's lymphomas. Arch Pathol Lab Med 116:1197, 1992

198. Pallesen G: The diagnostic significance of the CD30 (Ki-1) antigen. Histopathology 16:409, 1990

199. Agnarsson BA, Kadin ME: The immunophenotype of Reed-Sternberg cells. A study of 50 cases of Hodgkin's disease using fixed frozen tissues. Cancer 63:2083, 1989

200. Eckert F, Schmid U, Kaudewitz P, Burg G, Braun-Falco O: Follicular lymphoid hyperplasia of the skin with high content of Ki-1 positive lymphocytes. Am J Dermatopathol 11:345, 1989

201. Maeda K, Takahashi M: Characterization of skin infiltrating cells in adult T-cell leukaemia/lymphoma (ATLL): Clinical, histological and immunohistochemical studies on eight cases. Br J Dermatol 121:603, 1989

202. Hall PA, d'Ardenne AJ, Stansfeld AG: Paraffin section immunohistochemistry. II. Hodgkin's disease and large cell anaplastic (Ki1) lymphoma. Histopathology 13:161, 1988

203. Penny RJ, Blaustein JC, Longtine JA, Pinkus GS: Ki-1-positive large cell lymphomas, a heterogenous group of neoplasms. Morphologic, immunophenotypic, genotypic, and clinical features of 24 cases. Cancer 68:362, 1991

204. Burns BF, Dardick I: Ki-1–positive non-Hodgkin's lymphomas. An immunophenotypic, ultrastructural, and morphometric study. Am J Clin Pathol 93:327, 1990

205. Piris M, Brown DC, Gatter KC, Mason DY: CD30 expression in non-Hodgkin's lymphoma. Histopathol 17:211, 1990

206. Piris M, Gatter KC, Mason DY: CD30 expression in follicular lymphoma. Histopathology 18:25, 1991

207. Gianotti R, Alessi E, Cavicchini S, Berti E: Primary cutaneous pleomorphic T-cell lymphoma expressing CD30 antigen. Am J Dermatopathol 13:503, 1991

208. de Bruin PC, Beljaards RC, van Heerde P, Van Der Valk P, Noorduyn LA, Van Krieken JH, Kluin-Nelemans JC, Willemze R, Meijer CJ: Differences in clinical behaviour and immunophenotype between primary cutaneous and primary nodal anaplastic large cell lymphoma of T-cell or null cell phenotype. Histopathology 23:127, 1993

209. de Bruin PC, Noorduyn AL, van der Valk P, van Heerde P, van Diest PJ, van de Sandt MM, Ossenkoppele GJ, Meijer CJ: Noncutaneous T-cell lymphomas. Recognition of a lymphoma type (large cell anaplastic) with a relatively favorable prognosis. Cancer 71:2604, 1993

210. Noorduyn LA, de Bruin PC, van Heerde P, van de Sandt MM, Ossenkoppele GJ, Meijer CJ: Relation of CD30 expression to survival and morphology in large cell B cell lymphomas. J Clin Pathol 47:33, 1994

211. Pallesen G, Hamilton-Dutoit SJ: Ki-1 (CD30) antigen is regularly expressed by tumor cells of embryonal carcinoma. Am J Pathol 133:446, 1988

212. Mechtersheimer G, Moller P: Expression of Ki-1 antigen (CD30) in mesenchymal tumors. Cancer 66:1732, 1990

213. Ito K, Watanabe T, Horie R, Shiota M, Kawamura S, Mori S: High expression of the CD30 molecule in human decidual cells. Am J Pathol 145:276, 1994

214. Engert A, Burrows F, Jung W, Tazzari PL, Stein H, Pfreundschuh M, Diehl V, Thorpe P: Evaluation of ricin A chain-containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. Cancer Res 50:84, 1990

215. Falini B, Bolognesi A, Flenghi L, Tazzari PL, Broe MK, Stein H, Durkop H, Aversa F, Corneli P, Pizzolo G, Barbabietola G, Sabattini E, Pileri S, Martelli MF, Stirpe F: Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin. Lancet 339:1195, 1992

216. Pfreundschuh M, Pohl C, Berenbeck C, Schroeder J, Jung W, Schmits R, Tschiersch A, Diehl V, Gause A: Detection of a soluble form of the CD30 antigen in sera of patients with lymphoma, adult T-cell leukemia and infectious mononucleosis. Int J Cancer 45:869, 1990

217. Pizzolo G, Vinante F, Chilosi M, Dallenbach F, Josimovic-Alasevic O, Diamantstein T, Stein H: Serum levels of soluble CD30 molecule (Ki-1 antigen) in Hodgkin's disease: Relationship with disease activity and clinical stage. Br J Haematol 75:282, 1990

218. Gause A, Pohl C, Tschiersch A, Da Costa L, Jung W, Diehl V, Hasenclever D, Pfreundschuh M: Clinical significance of soluble CD30 antigen in the sera of patients with untreated Hodgkin's disease. Blood 77:1983, 1991

219. Nadali G, Vinante F, Ambrosetti A, Todeschini G, Veneri D, Zanotti R, Meneghini V, Ricetti MM, Benedetti F, Vassanelli A,

Perona G, Chilosi M, Menestrina F, Fiacchini M, Stein H, Pizzolo G: Serum levels of soluble CD30 are elevated in the majority of untreated patients with Hodgkin's disease and correlate with clinical features and prognosis. J Clin Oncol 12:793, 1994

220. Gause A, Jung W, Keymis S, Schobert I, Scholz R, Schmits R, Diehl V, Pohl C, Hasenclever D, Pfreundschuh M: The clinical significance of cytokines and soluble forms of membrane-derived activation antigens in the serum of patients with Hodgkin's disease. Leuk Lymphoma 7:439, 1992

221. Gause A, Jung W, Schmits R, Tschiersch A, Scholz R, Pohl C, Hasenclever D, Diehl V, Pfreundschuh M: Soluble CD8, CD25 and CD30 antigens as prognostic markers in patients with untreated Hodgkin's lymphoma. Ann Oncol 4:49, 1992

222. Gruss H-J, DaSilva N, Hu Z-B, Uphoff CC, Goodwin RG, Drexler HG: Expression and regulation of CD30 ligand and CD30 in human leukemia-lymphoma cell lines. Leukemia 8:2083, 1994

223. Alzona M, Jäck H-M, Fisher R, Ellis TM: CD30 defines a subset of activated human T cells that produce IFN-γ and IL-5 and exhibit enhanced B cell helper activity. J Immunol 153:2861, 1994

224. Kadin ME, Sako D, Berliner N, Franklin W, Woda B, Borowitz M, Ireland K, Schweid A, Herzog P, Lange B: Childhood Ki-1 lymphoma presenting with skin lesions and peripheral lymphadenopathy. Blood 68:1042, 1986

225. Agnarsson BA, Kadin ME: Ki-1 positive large cell lymphoma. A morphologic and immunologic study of 19 cases. Am J Surg Pathol 12:264, 1988

226. Delsol G, Al Saati T, Gatter KC, Gerdes J, Schwarting R, Caveriviere P, Rigal-Huguet F, Robert A, Stein H, Mason DY: Coexpression of epithelial membrane antigen (EMA), Ki-1, and interleukin-2 receptor by anaplastic large cell lymphomas. Diagnostic value in so-called malignant histiocytosis. Am J Pathol 130:59, 1988

227. Kinney MC, Glick AD, Stein H, Collins RD: Comparison of anaplastic large cell Ki-1 lymphomas and microvillous lymphomas in their immunologic and ultrastructural features. Am J Surg Pathol 14:1047, 1990

228. Mason DY, Bastard C, Rimokh R, Dastugue N, Huret JL, Kristoffersson U, Magaud JP, Nezelof C, Tilly H, Vannier JP, Hemet J, Warnke R: CD30-positive large cell lymphomas ('Ki-1 lymphoma') are associated with a chromosomal translocation involving 5q35. Br J Haematol 74:161, 1990

229. Chott A, Kaserer K, Augustin I, Vesely M, Heinz R, Oehlinger W, Hanak H, Radaszkiewicz T: Ki-1–positive large cell lymphoma. A clinicopathologic study of 41 cases. Am J Surg Pathol 14:439, 1990

230. Le Beau MM, Bitter MA, Larson RA, Doane LA, Ellis ED, Franklin WA, Rubin CM, Kadin ME, Vardiman JW: The t(2;5)(p23;q35): A recurring chromosomal abnormality in Ki-1–positive anaplastic large cell lymphoma. Leukemia 3:866, 1989

231. Strickler JG, Michie SA, Warnke RA, Dorfman RF: The "syncytial variant" of nodular sclerosing Hodgkin's disease. Am J Surg Pathol 10:470, 1986

232. Kadin ME: Primary Ki-1-positive anaplastic large-cell lymphoma: A distinct clinicopathologic entity. Ann Oncol 5:25, 1994

233. Kadin ME: Ki-1/CD30$^+$ (anaplastic) large-cell lymphoma: Maturation of a clinicopathologic entity with prospects of effective therapy. J Clin Oncol 12:884, 1994

234. Anagnostopoulos I, Herbst H, Niedobitek G, Stein H: Demonstration of monoclonal EBV genomes in Hodgkin's disease and Ki-1–positive anaplastic large cell lymphoma by combined Southern blot and in situ hybridization. Blood 74:810, 1989

235. Herbst H, Dallenbach F, Hummel M, Niedobitek G, Finn T, Young LS, Rowe M, Müller-Lantzsch N, Stein H: Epstein-Barr virus DNA and latent gene products in Ki-1 (CD30)–positive anaplastic large cell lymphomas. Blood 78:2666, 1991

236. Ross CW, Schlegelmilch JA, Grogan TM, Weiss LM, Schnitzer B, Hanson CA: Detection of Epstein-Barr virus genome in Ki-1 (CD30)-positive, large-cell anaplastic lymphomas using the polymerase chain reaction. Am J Pathol 141:457, 1992

237. Lopategui JR, Gaffey MJ, Chan JKC, Frierson HF Jr, Sun L-H, Bellafiore FJ, Chang KL, Weiss LW: Infrequent association of Epstein-Barr virus with CD30-positive anaplastic large cell lymphomas from American and Asian patients. Am J Surg Pathol 19:42, 1995

238. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT: Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263:1281, 1994

239. Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T, Mori S: Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. Oncogene 9:1567, 1994

240. Ladanyi M, Cavalchire G, Morris SW, Downing J, Filippa DA: Reverse transcriptase polymerase chain reaction for the Ki-1 anaplastic large cell lymphoma-associated t(2;5) translocation in Hodgkin's disease. Am J Pathol 145:1296, 1994

241. Orscheschek K, Merz H, Hell J, Binder T, Bartels H, Feller AC: Large-cell anaplastic lymphoma-specific translocation (t:2;5)(p23;q35) in Hodgkin's disease: Indication of a common pathogenesis? Lancet 345:87, 1995

242. Bullrich F, Morris SW, Hummel M, Pileri S, Stein H, Croce CM: Nucleophosmin (NPM) gene rearrangements in Ki-1-positive lymphomas. Cancer Res 54:2873, 1994

243. Inghirami G, Macri L, Cesarman E, Chadburn A, Zhong J, Knowles DM: Molecular characterization of CD30$^+$ anaplastic large-cell lymphoma: High frequency of c-myc protooncogene activation. Blood 83:3581, 1994

244. Ledbetter JA, Shu G, Gallagher M, Clark EA: Augmentation of normal and malignant B cell proliferation by monoclonal antibody to the B cell-specific antigen BP50 (CD$_w$40). J Immunol 138:788, 1987

245. Uckun FM, Gajl-Peczalska K, Myers DE, Jaszcz W, Haissig S, Ledbetter JA: Temporal association of CD40 antigen expression with discrete stages of human B-cell ontogeny and the efficacy of anti-CD40 immunotoxins against clonogenic B-lineage acute lymphoblastic leukemia as well as B-lineage non-Hodgkin's lymphoma cells. Blood 76:2449, 1990

246. Galy AHM, Spits H: CD40 is functionally expressed on human thymic epithelial cells. J Immunol 149:775, 1992

247. Gauchat J-F, Henchoz S, Mazzei G, Aubry J-P, Brunner T, Blasey H, Life P, Talabot D, Flores-Romo L, Thompson J, Kishi K, Butterfield J, Dahinden C, Bonnefoy J-Y: Induction of human IgE synthesis in B cells by mast cells and basophils. Nature 365:340, 1993

248. Lane P, Brocker T, Hubele S, Padovan E, Lanzavecchia A, McConnell F: Soluble CD40 ligand can replace the normal T cell-derived CD40 ligand signal to B cells in T cell-dependent activation. J Exp Med 177:1209, 1993

249. Armitage RJ, Maliszewski CR, Alderson MR, Grabstein KH, Spriggs MK, Fanslow WC: CD40L: A multi-functional ligand. Semin Immunol 5:401, 1993

250. Armitage RJ, Macduff BM, Spriggs MK, Fanslow WC: Human B cell proliferation and Ig secretion induced by recombinant CD40 ligand are modulated by soluble cytokines. J Immunol 150:3671, 1993

251. Liu Y-J, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennon ICM: Mechanism of antigen-driven selection in germinal centres. Nature 342:929, 1989

252. Holder MJ, Wang H, Milner AE, Casamayor M, Armitage R, Spriggs MK, Fanslow WC, MacLennan ICM, Gregory CD, Gor-

don J: Suppression of apoptosis in normal and neoplastic human B lymphocytes by CD40 ligand is independent of Bcl-2 induction. Eur J Immunol 23:2368, 1993

253. Clark EA, Ledbetter JA: Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50. Proc Natl Acad Sci USA 83:4494, 1986

254. Gordon J, Millsum MJ, Guy GR, Ledbetter JA: Resting B lymphocytes can be triggered directly through the CDw40 (Bp50) antigen. J Immunol 140:1425, 1988

255. Jabara HH, Fu SM, Geha RS, Vercelli D: CD40 and IgE: Synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. J Exp Med 172:1861, 1990

256. Alderson MR, Armitage RJ, Tough TW, Strockbine L, Fanslow WC, Spriggs MK: CD40 expression by human monocytes: Regulation by cytokines and activation of monocytes by the ligand for CD40. J Exp Med 178:669, 1993

257. Carbone A, Gloghini A, Gattei V, Aldinucci D, Deagan M, De Paoli P, Zagonel V, Pinto A: Expression of functional CD40 antigen on Reed-Sternberg cells and Hodgkin's disease cell lines. Blood 85:780, 1995

258. Gruss H-J, Hirschstein D, Wright B, Ulrich D, Caligiuri MA, Strockbine L, Armitage RJ, Dower SK: Expression and function of CD40 on Hodgkin and Reed-Sternberg cells and the possible relevance for Hodgkin's disease. Blood 84:2305, 1994

259. Kennedy IC, Hart DN, Colls BM, Nimmo JC, Willis DA, Angus HB: Nodular sclerosing, mixed cellularity and lymphocyte-depleted variants of Hodgkin's disease are probable dendritic cell malignancies. Clin Exp Immunol 76:324, 1989

260. O'Grady JT, Stewart S, Lowrey J, Howie SE, Krajewski AS: CD40 expression in Hodgkin's disease. Am J Pathol 144:21, 1994

261. Pizzolo G, Vinante F, Nadali G, Ricetti MM, Morosato L, Marrocchella R, Vincenzi C, Semenzato G, Chilosi M: ICAM-1 tissue overexpression associated with increased serum levels of its soluble form in Hodgkin's disease. Br J Haematol 84:161, 1993

262. Delabie J, Ceuppens JL, Vandenberghe P, de Boer M, Coorevits L, De Wolf-Peeters C: The B7/BB1 antigen is expressed by Reed-Sternberg cells of Hodgkin's disease and contributes to the stimulating capacity of Hodgkin's disease-derived cell lines. Blood 82:2845, 1993

263. Munro JM, Freedman AS, Aster JC, Gribben JG, Lee NC, Rhynhart KK, Banchereau J, Nadler LM: In vivo expression of the B7 costimulatory molecule by subsets of antigen-presenting cells and malignant cells of Hodgkin's disease. Blood 83:793, 1994

264. Gruss HJ, Dölken G, Brach MA, Mertelsmann R, Herrmann F: Serum levels of circulating ICAM-1 are increased in Hodgkin's disease. Leukemia 7:1245, 1993

265. Ruco LP, Pomponi D, Pigott R, Stoppacciaro A, Monardo F, Uccini S, Boraschi D, Tagliabue A, Santoni A, Dejana E, Mantovani A, Baroni CD: Cytokine production (IL-1 alpha, IL-1 beta, and TNF alpha) and endothelial cell activation (ELAM-1 and HLA-DR) in reactive lymphadenitis, Hodgkin's disease, and in non-Hodgkin's lymphomas. An immunocytochemical study. Am J Pathol 137:1163, 1990

266. Ruco LP, Pomponi D, Pigott R, Gearing AJ, Baiocchini A, Baroni CD: Expression and cell distribution of the intercellular adhesion molecule, vascular cell adhesion molecule, endothelial leukocyte adhesion molecule, and endothelial cell adhesion molecule (CD31) in reactive human lymph nodes and in Hodgkin's disease. Am J Pathol 140:1337, 1992

267. Paulie S, Ehlin-Henriksson B, Mellstedt H, Koho H, Ben-Aissa H, Perlmann P: A p50 surface antigen restricted to human urinary bladder carcinomas and B lymphocytes. Cancer Immunol Immunother 20:23, 1985

268. Clark EA: CD40: A cytokine receptor in search of a ligand. Tissue Antigens 36:33, 1990

269. Uckun FM: Regulation of human B-cell ontogeny. Blood 76:1908, 1990

270. Valle A, Zuber CE, Defrance T, Djossou O, DeRie M, Banchereau J: Activation of human B lymphocytes through CD40 and interleukin 4. Eur J Immunol 19:1463, 1989

271. Banchereau J, de Paoli P, Vallé A, Garcia E, Rousset F: Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. Science 251:70, 1991

272. Banchereau J, Rousset F: Growing human B lymphocytes in the CD40 system. Nature 353:678, 1991

273. Rousset F, Garcia E, Banchereau J: Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. J Exp Med 173:705, 1991

274. Rousset F, Garcia E, Defrance T, Péronne C, Vezzio N, Hsu D-H, Kastelein R, Moore KW, Banchereau J: Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 89:1890, 1992

275. Zhang K, Clark EA, Saxon A: CD40 stimulation provides and IFN-γ–independent and IL-4–dependent differentiation directly to human B cells for IgE production. J Immunol 146:1836, 1991

276. Gascan H, Gauchat J-F, Aversa G, van Vlasselaer P, de Vries JE: Anti-CD40 monoclonal antibodies or $CD4^+$ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signalling pathways. J Immunol 147:8, 1991

277. Liu YJ, Mason DY, Johnson GD, Abbot S, Gregory CD, Hardie DL, Gordon J, Mac Lennan IC: Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. Eur J Immunol 21:1905, 1991

278. Conley ME: Molecular approaches to analysis of X-linked immunodeficiencies. Annu Rev Immunol 10:215, 1992

279. Notarangelo LD, Duse M, Ugazio AG: Immunodeficiency with hyper-IgM (HIM). Immunodefic Rev 3:101, 1992

280. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, Bedell MA, Edelhoff S, Disteche CM, Simoneaux DK, Fanslow WC, Belmont J, Spriggs MK: CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science 259:990, 1993

281. Fluckiger AC, Rossi JF, Bussel A, Bryon P, Banchereau J, Defrance T: Responsiveness of chronic lymphocytic leukemia B cells activated via surface Igs or CD40 to B-cell tropic factors. Blood 80:3173, 1992

282. Delabie J, Shipman R, Bruggen J, De Strooper B, van Leuven F, Tarcsay L, Cerletti N, Odink K, Diehl V, Bilbe G, De Wolf-Peeters C: Expression of the novel intermediate filament-associated protein restin in Hodgkin's disease and anaplastic large-cell lymphoma. Blood 80:2891, 1992

283. Merz H, Houssiau FA, Orscheschek K, Renauld JC, Fliedner A, Herin M, Noel H, Kadin M, Mueller-Hermelink HK, Van Snick J, Feller AC: Interleukin-9 expression in human malignant lymphomas: Unique association with Hodgkin's disease and large cell anaplastic lymphoma. Blood 78:1311, 1991

284. Pinto A, Gloghini A, Gattei V, Aldinucci D, Zagonel V, Carbone A: Expression of the c-*kit* receptor in human lymphomas is restricted to Hodgkin's disease and $CD30^+$ anaplastic large cell lymphomas. Blood 83:785, 1994

285. Lederman S, Yellin MJ, Inghirami G, Lee JJ, Knowles DM, Chess L: Molecular interactions mediating T-B lymphocyte collaboration in human lymphoid follicles. J Immunol 149:3817, 1992

286. Inghirami G, Lederman S, Yellin MJ, Chadburn A, Chess L, Knowles DM: Phenotypic and functional characterization of T-BAM (CD40 ligand) T-cell non-Hodgkin's lymphoma. Blood 84:866, 1994

287. Ashwell JD, Longo DL, Bridges SH: T-cell tumor elimina-

tion as a result of T-cell receptor-mediated activation. Science 237:61, 1987

288. Bridges SH, Kruisbeek AM, Longo DL: Selective in vivo antitumor effects of monoclonal anti-I-A antibody on B cell lymphoma. J Immunol 139:4242, 1987

289. Page DM, DeFranco AL: Role of phosphoinositide-derived second messengers in mediating anti-IgM-induced growth arrest of WEHI-231 B lymphoma cells. J Immunol 140:3717, 1988

290. Beckwith M, Longo DL, O'Connell CD, Moratz CM, Urba WJ: Phorbol ester-induced, cell-cycle-specific, growth inhibition of human B-lymphoma cell lines. J Natl Cancer Inst 82:501, 1990

291. Funakoshi S, Longo DL, Beckwith M, Conley DK, Tsarfaty G, Tsarfaty I, Armitage RJ, Fanslow WC, Spriggs MK, Murphy WJ: Inhibition of human B-cell lymphoma growth by CD40 stimulation. Blood 83:2787, 1994

292. Sumimoto S-I, Heike T, Kanazashi S-I, Shintaku N, Jung E-Y, Hata D, Katamura K, Mayumi M: Involvement of LFA-1/intracellular adhesion molecule-1–dependent cell adhesion in CD40-mediated inhibition of human B lymphoma cell death induced by surface IgM crosslinking. J Immunol 153:2488, 1994

293. Murphy WJ, Funakoshi S, Beckwith M, Conley DK, Armitage RJ, Longo DL: Antibodies to CD40 promote normal human B-cell engraftment and inhibit B cell lymphomagenesis in vivo. Proc Natl Acad Sci USA (in press)

294. Ellis RE, Yuan JY, Horvitz HR: Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663, 1991

295. Raff MC: Social controls on cell survival and cell death. Nature 356:397, 1992

296. Wyllie AH, Kerr JF, Currie AR: Cell death: The significance of apoptosis. Int Rev Cytol 68:251, 1980

297. Walker NI, Harmon BV, Gobe GC, Kerr JF: Patterns of cell death. Methods Achiev Exp Pathol 13:18, 1988

298. Watanabe-Fukunaga R, Brannan CI, Itoh N, Yonehara S, Copeland NG, Jenkins NA, Nagata S: The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. J Immunol 148:1274, 1992

299. Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S: Lethal effect of the anti-Fas antibody in mice. Nature 364:806, 1993

300. Owen-Schaub LB, Yonehara S, Crump WL III, Grimm EA: DNA fragmentation and cell death is selectively triggered in activated human lymphocytes by Fas antigen engagement. Cell Immunol 140:197, 1992

301. Daniel PT, Krammer PH: Activation induces sensitivity toward APO-1 (CD95)-mediated apoptosis in human B cells. J Immunol 152:5624, 1994

302. Debatin KM, Goldmann CK, Bamford R, Waldmann TA, Krammer PH: Monoclonal-antibody–mediated apoptosis in adult T-cell leukaemia. Lancet 335:497, 1990

303. Kobayashi N, Hamamoto Y, Yamamoto N, Ishii A, Yonehara M, Yonehara S: Anti-Fas monoclonal antibody is cytocidal to human immunodeficiency virus-infected cells without augmenting viral replication. Proc Natl Acad Sci USA 87:9620, 1990

304. Falk MH, Trauth BC, Debatin KM, Klas C, Gregory CD, Rickinson AB, Calender A, Lenoir GM, Ellwart JW, Krammer PH, Bornkamm GW: Expression of the APO-1 antigen in Burkitt lymphoma cell lines correlates with a shift towards a lymphoblastoid phenotype. Blood 79:3300, 1992

305. Mapara MY, Bargou R, Zugck C, Döhner H, Ustaoglu F, Jonker RR, Krammer PH, Dörken B: APO-1 mediated apoptosis or proliferation in human chronic B lymphocytic leukemia: Correlation with bcl-2 oncogene expression. Eur J Immunol 23:702, 1993

306. Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ: Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334, 1990

307. Reed JC: Bcl-2 and the regulation of programmed cell death. J Cell Biol 124:1, 1994

308. Möller P, Henne C, Leithauser F, Eichelmann A, Schmidt A, Bruderlein S, Dhein J, Krammer PH: Coregulation of the APO-1 antigen with intercellular adhesion molecule-1 (CD54) in tonsillar B cells and coordinate expression in follicular center B cells and in follicle center and mediastinal B-cell lymphomas. Blood 81:2067, 1993

309. Owen-Schaub LB, Meterissian S, Ford RJ: Fas/APO-1 expression and function on malignant cells of hematologic and nonhematologic origin. J Immunother 14:234, 1993

310. Klas C, Debatin K-M, Jonker RR, Krammer PH: Activation interferes with the APO-1 pathway in mature human T cells. Int Immunol 5:625, 1993

311. Rouvier E, Luciani M-F, Golstein P: Fas involvement in Ca^{2+}-independent T cell-mediated cytotoxicity. J Exp Med 177:195, 1993

312. Alderson MR, Tough TW, Braddy S, Davis-Smith T, Roux E, Schooley K, Miller RE, Lynch DH: Regulation of apoptosis and T cell activation by Fas-specific mAb. Int Immunol 6:1799, 1994

313. Kondo E, Yoshino T, Yamadori I, Matsuo Y, Kawasaki N, Minowada J, Akagi T: Expression of Bcl-2 and Fas antigen in non-Hodgkin's lymphomas. Am J Pathol 145:330, 1994

314. Kotani T, Aratake Y, Kondo S, Tamura K, Ohtaki S: Expression of functional Fas antigen on adult T-cell leukemia. Leuk Res 18:305, 1994

315. Vignaux F, Golstein P: Fas-based lymphocyte-mediated cytotoxicity against syngeneic activated lymphocytes: A regulatory pathway: Eur J Immunol 24:923, 1994

316. Crispe IN: Fatal interactions: Fas-induced apoptosis of mature T cells. Immunity 1:347, 1994

317. Kwon BS, Kim GS, Prystowsky MB, Lancki DW, Sabath DE, Pan JL, Weissman SM: Isolation and initial characterization of multiple species of T-lymphocyte subset cDNA clones. Proc Natl Acad Sci USA 84:2896, 1987

318. Schwarz H, Tuckwell J, Lotz M: A receptor induced by lymphocyte activation (Ila)—A new member of the human nerve-growth-factor tumor-necrosis-factor receptor family. Gene 134:295, 1993

319. Pollok KE, Kim Y-J, Zhou Z, Hurtado J, Kim KK, Pickard RT, Kwon BS: Inducible T cell antigen 4-1BB. Analysis of expression and function. J Immunol 150:771, 1993

320. Chalupny NJ, Peach R, Hollenbaugh D, Ledbetter JA, Farr AG, Aruffo A: T-cell activation molecule 4-1BB binds to extracellular matrix proteins. Proc Natl Acad Sci USA 89:10360, 1992

321. Paterson DJ, Jefferies WA, Green JR, Brandon MR, Corthesy P, Puklavec M, Williams AF: Antigens of activated rat T lymphocytes including a molecule of 50,000 M, detected only on CD4 positive T blasts. Mol Immunol 24:1281, 1987

322. Mallett S, Fossum S, Barclay AN: Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes—A molecule related to nerve growth factor receptor. EMBO J 9:1063, 1990

323. Calderhead DM, Buhlmann JE, van den Eertwegh AJM, Claassen E, Noelle RJ, Fell HP: Cloning of mouse OX40: A T cell activation marker that may mediate T-B cell interactions. J Immunol 151:5261, 1993

324. Latza U, Dürkop H, Schnittger S, Ringeling J, Eitelbach F, Hummel M, Fonatsch C, Stein H: The human OX40 homolog: cDNA structure, expression and chromosomal assignment of the ACT35 antigen. Eur J Immunol 24:677, 1994

325. Tozawa H, Andoh S, Takayama Y, Tanaka Y, Lee B, Nakamura H, Hayami M, Hinuma Y: Species-dependent antigenicity of the 34-kDa glycoprotein found on the membrane of various primate lymphocytes transformed by human T-cell leukemia virus type-I

(HTLV-I) and simian T-cell leukemia virus (STLV-I). Int J Cancer 41:231, 1988

326. Miura S, Ohtani K, Numata N, Niki M, Ohbo K, Ina Y, Gojobori T, Tanaka Y, Tozawa H, Nakamura M, Sugamura K: Molecular cloning and characterization of a novel glycoprotein, gp34, that is specifically induced by the human T-cell leukemia virus type I transactivator $p40^{tax}$. Mol Cell Biol 11:1313, 1991

327. Ruggiero V, Latham K, Baglioni C: Cytostatic and cytotoxic activity of tumor necrosis factor on human cancer cells. J Immunol 138:2711, 1987

328. Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YC, Mathison J, Ulevitch R, Cerami A: Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature 316:552, 1985

329. Bendtzen K: Interleukin 1, interleukin 6 and tumor necrosis factor in infection, inflammation and immunity. Immunol Lett 19:183, 1988

330. Paul NL, Ruddle NH: Lymphotoxin. Annu Rev Immunol 6:407, 1988

331. Tracey KJ, Cerami A: Tumor necrosis factor—A pleiotropic cytokine and therapeutic target. Annu Rev Med 45:491, 1994

332. Scheurich P, Thoma B, Ucer U, Pfizenmaier K: Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-alpha: Induction of TNF receptors on human T cells and TNF-alpha–mediated enhancement of T cell responses. J Immunol 138:1786, 1987

333. Shchelkunov SN, Blinov VM, Sandakhchiev LS: Genes of variola and vaccinia viruses necessary to overcome the host protective mechanisms. FEBS Lett 319:80, 1993

334. Hu FQ, Pickup DJ: Transcription of the terminal loop region of vaccinia virus DNA is initiated from the telomere sequences directing DNA resolution. Virology 181:716, 1991

335. Gruss H-J, Brach MA, Drexler HG, Bonifer R, Mertelsmann RH, Herrmann F: Expression of cytokine genes, cytokine receptor genes, and transcription factors in cultured Hodgkin and Reed-Sternberg cells. Cancer Res 52:3353, 1992

336. Hsu PL, Hsu SM: Production of tumor necrosis factor-alpha and lymphotoxin by cells of Hodgkin's neoplastic cell lines HDLM-1 and KM-H2. Am J Pathol 135:735, 1989

337. Kretschmer C, Jones DB, Morrison K, Schluter C, Feist W, Ulmer AJ, Arnoldi J, Matthes J, Diamantstein T, Flad HD, Gerdes J: Tumor necrosis factor alpha and lymphotoxin production in Hodgkin's disease. Am J Pathol 137:341, 1990

338. Klein S, Jücker M, Diehl V, Tesch H: Production of multiple cytokines by Hodgkin's disease derived cell lines. Hematol Oncol 10:319, 1992

339. Sappino AP, Seelentag W, Pelte MF, Alberto P, Vassalli P: Tumor necrosis factor/cachectin and lymphotoxin gene expression in lymph nodes from lymphoma patients. Blood 75:958, 1990

340. Xerri L, Birg F, Guigou V, Bouabdallah R, Poizot-Martin I, Hassoun J: In situ expression of the IL-1–alpha and TNF-alpha genes by Reed-Sternberg cells in Hodgkin's disease. Int J Cancer 50:689, 1992

341. Ryffel B, Brockhaus M, Durmuller U, Gudat F: Tumor necrosis factor receptors in lymphoid tissues and lymphomas. Source and site of action of tumor necrosis factor alpha. Am J Pathol 139:7, 1991

342. Gruss HJ, Brach MA, Herrmann F: Involvement of cytokines in Hodgkin's disease, in Abraham NG, Konwalinka G, Marks P, Sachs L, Tauasser M (eds): Molecular Biology of Hematopoiesis, vol 2. Andover, UK, Intercept Ltd, 1992, p 217

343. Dayer JM, Beutler B, Cerami A: Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J Exp Med 162:2163, 1985

344. Gruss HJ, Dölken G, Brach MA, Mertelsmann R, Herrmann F: The significance of serum levels of soluble 60kDa receptors for tumor necrosis factor in patients with Hodgkin's disease. Leukemia 7:1339, 1993

345. Slivnick DJ, Nawrocki JF, Fisher RI: Immunology and cellular biology of Hodgkin's disease. Hematol Oncol Clin North Am 3:205, 1989

346. Slivnick DJ, Ellis TM, Nawrocki JF, Fisher RI: The impact of Hodgkin's disease on the immune system. Semin Oncol 17:673, 1990

347. Ruco LP, Stoppacciaro A, Pomponi D, Boraschi D, Santoni A, Tagliabue A, Uccini S, Baroni CD: Immunoreactivity for IL-1 beta and TNF alpha in human lymphoid and nonlymphoid tissues. Am J Pathol 135:889, 1989

348. McCall JL, Yun K, Funamoto S, Parry BR: In vivo immunohistochemical identification of tumor necrosis factor/cachectin in human lymphoid tissue. Am J Pathol 135:421, 1989

349. Krönke M, Hensel G, Schluter C, Scheurich P, Schutze S, Pfizenmaier K: Tumor necrosis factor and lymphotoxin gene expression in human tumor cell lines. Cancer Res 48:5417, 1988

350. Kehrl JH, Miller A, Fauci AS: Effect of tumor necrosis factor α on mitogen-activated human B cells. J Exp Med 166:786, 1987

351. Digel W, Stefanic M, Schoniger W, Buck C, Raghavachar A, Frickhofen N, Heimpel H, Porzsolt F: Tumor necrosis factor induces proliferation of neoplastic B cells from chronic lymphocytic leukemia. Blood 73:1242, 1989

352. Gibbons DL, Rowe M, Cope AP, Feldmann M, Brennan FM: Lymphotoxin acts as an autocrine growth factor for Epstein-Barr virus-transformed B cells and differential Burkitt lymphoma cell lines. Eur J Immunol 24:1879, 1994